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Délivré par :

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A ma mère,

qui n'a jamais cessé de croire en moi !

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Par définition une thèse c'est : « une thèse est un mémoire résumant un travail de recherche universitaire, soutenu devant un jury par un étudiant afin d'obtenir un diplôme ou un grade universitaire » (Wikipédia).

Cependant, une thèse est bien plus que ça, c'est à la fois un partage, certes beaucoup scientifique, mais aussi humain. En effet, la réalisation d'un tel projet ne se fait pas seule mais en équipe, c'est pourquoi cette partie risque d'être un peu longue. Merci à vous d'avoir la patience et / ou l'intérêt de lire ce passage.

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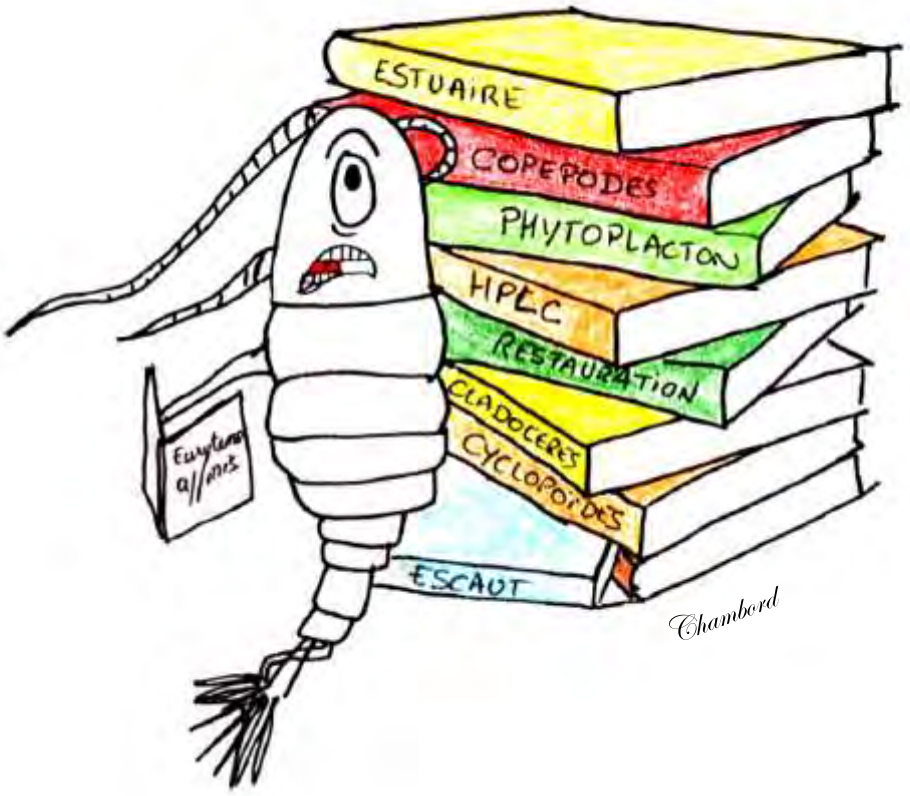
I. CHAPITRE I :

Introduction



La gestion des ressources en eau et depuis quelques décennies une préoccupation majeure au niveau mondial (e.g. Ashley et al., 2006, Vörösmarty et al., 2010 ; Conseil mondial de l'eau, 2016). Depuis plusieurs années, les recherches en hydro-écologie et en gestion intégrée de la ressource en eau ont pour but la réduction de l'impact des activtés anthropiques sur le fonctionnement écologique des milieux aquatiques. Une bonne compréhension du fonctionnement écologique et donc indispensable pour anticiper l'effet des futurs aménagements sur la qualité de l'eau. La restauration des sytèmes aquatiques concerne une grande diversité des personnes impliqées dans diverses activités : agriculture, industrie, navigation et ports, administration, recherche scientifique, associations diverses (pêche, protection de la nature).

Les zones côtiers et estuariennes constituent les écosystèmes les plus productifs de la planète (Costanza et al., 1997). A l'interface terre-mer, les estuaires représentent des enjeux contradictoires en raison de leur position stratégique. Ils constituent des écosystèmes particuliers, tout en concentrant des activités socio-économiques considérables (activités portuaires et industrielles (Elliot et Ducrotoy, 1991), agricoles et de loisirs). A ce tirtre, et afin de favoriser l'implantation de l'activité inductriells les estuaires ont souvent fait l'objet de nombreux aménagements (portuaires, inductriels, chenalisation). La pollution et la fragilisation de ces milieux et des écosystèmes en particulier accroît avec le développement des activités qui s'accompagne d'un accroissement de la population et des rejets anthropiques.

I.1 Le milieu estuarien

I.2 Le concept « estuaire »

Du latin *aestuarium*, ce terme fait référence aux milieux où les eaux continentales sont soumises à l'influence de la marée avant de rejoindre la mer. D'un point de vue très réducteur il est courant d'assimiler, l'estuaire à l'embouchure d'un cours d'eau se jetant dans la mer. Sa définition est beaucoup plus complexe et fait encore l'objet de débats et de nouvelles propositions (Elliott et McLusky, 2002 ; Potter et al., 2010). La capacité à réunir les notions liées à la distribution de salinité et de densité aux caractéristiques des processus de circulation et de mélange, et aux

limites contrôlant la distribution et le mouvement des mélanges d'eaux, font de la formulation de Pritchard (1967) une des plus reconnues :

« Une masse d'eau côtière semi-close qui est en libre connexion avec la mer et dans laquelle l'eau de mer est mélangée à une quantité mesurable d'eau douce provenant du drainage continental.» (Pritchard, 1967).

Toutefois, différents auteurs ont souligné d'autres aspects du milieu estuarien. Les effets des marées sont précisés par Dionne (1963) puis la définition de Pritchard (1967) est reprise et enrichie par Fairbridge (1980).

« Un estuaire est un bras de mer pénétrant une vallée fluviale jusqu'à la limite amont de propagation de la marée, habituellement divisé en trois secteurs : (a) un bas estuaire ou estuaire marin ; (b) un estuaire moyen, sujet à des importants mélanges entre deux eaux douces et eaux salées ; (c) un haut estuaire ou estuaire fluvial, caractérisé par l'eau douce mais sujet aux actions quotidiennes de la marée. Les limites entre ces trois secteurs sont variables et dépendantes des constantes variations du débit fluvial.» (Fairbridge, 1980).

Perillo (1995) vient élargir cette définition en faisant référence aux marées et en y ajoutant la biocénose.

« Un estuaire est une masse d'eau semi-fermée qui s'étend jusqu'à la limite des marées, dans laquelle l'eau de mer entrant par une ou plusieurs connexions libres avec la mer ouverte ou avec quelconque autre masse d'eau salée côtière, est significativement diluée avec l'eau douce dérivant par drainage continental et qui peut contenir des espèces euryhalines pendant une partie ou l'ensemble de leur cycle de vie. » (Perillo, 1995).

Plus récemment, Potter et al. (2010) ajoutent la possibilité d'une rupture saisonnière de la connexion à la mer et d'une hypersalinisation quand les pertes d'eau par évaporation sont fortes et les apports d'eau douce négligeables. Avec cette définition, le concept d'estuaire s'étend et permet de connecter des estuaires australiens ou sud-africains.

A ceci s'ajoutent des visions juridiques, socio-économiques et administratives (Elliott et McLusky, 2002), ainsi que diverses classifications des systèmes estuariens basées sur des critères physiques (Dyer, 1997), géomorphologiques ou hydrologiques.

En résumé les estuaires sont des milieux en constante évolution, très dynamiques (échanges de flux terre-mer) et très complexes (multi-échelles et les couplages complexes entre ces processus physiques, chimiques et biologiques).

I.2.1 Les spécificités des milieux estuariens

Le phénomène de marée, s'exerçant de façon cyclique sur certaines côtes est aujourd'hui bien connu. La marée est causée par l'effet conjugué des forces de gravitation dues à la lune et au soleil, et de la force d'inertie due à la révolution de la terre autour du centre de gravité du système terre-lune. Le débit fluvial, quant à lui, est principalement dépendant de la pluviométrie ainsi que des caractéristiques du bassin versant (taille, présence de barrages, etc). Ces deux forces – marée et débit fluvial – vont s'opposer aux niveaux desestuaires. La physicochimie du milieu estuarien et la distribution spatiale des communautés estuariennes vont être conditionnées en générale par l'émulation des forces hydrauliques provenant du débit fluvial et de la marée.

L'un des premiers facteurs environnementaux à être influencé par cette opposition de forces est la salinité. En effet, la salinité dans les estuaires décroît selon un gradient aval-amont, c'est ainsi que différentes zones ont été définies. Trois zones estuariennes sont définies en fonction de la salinité (Reid, 1961 ; Mouny, Dauvin et al., 1996) : la zone oligohaline, de 0,5 à 5 ; la zone mesohaline, de 5 à 18 ; la zone polyhaline, de 18 à 30. Le mélange des eaux marines et continentales dépend des caractéristiques géomorphologiques des estuaires. Ainsi, en plus d'un gradient de salinité longitudinal, la stratification verticale de la salinité peut être plus ou moins importante, l'eau salée étant plus dense que l'eau douce.

Basé sur les différents types de circulation des eaux estuariennes, Pritchard (1995), met en place la première classification significative des estuaires, on distingue ainsi les estuaires du plus stratifié au plus homogène (Fig.1).

- Type A : **Estuaire sans mélange ou complètement stratifié**, où les flux dominant sont issus de l'eau douce.
- Type B : **Estuaire à coin salé**, où les gradients verticaux sont importants. Volume d'eau salée < volume d'eau douce. Ce type de comportement correspond aux estuaires à faible marnage, dans lesquels la stratification

verticale est maximale. L'eau douce s'écoule en surface. En profondeur, la masse d'eau forme un coin salé pratiquement stationnaire (Dyer, 1986). La zone de transition entre les deux couches est restreinte avec un fort gradient, rendant difficile le mélange des masses d'eau.

- Type C : **Estuaire partiellement mélangé**. Volume d'eau salée > volume d'eau douce, créant des courants plus forts et des excursions, longitudinales et verticales, plus importantes, des masses d'eau.
- Type D : **Estuaire homogène**, sans gradient de salinité. Les marées sont plus prépondérantes par rapport à la circulation de densité, le mélange se fait sur toute la colonne d'eau. En général, ce type de mélange correspond aux estuaires peu profonds possédant un fort marnage

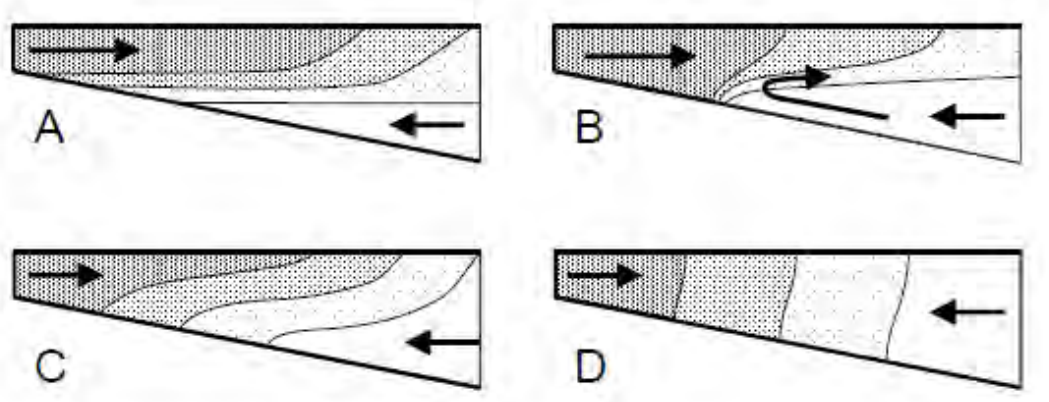


Figure 1 : Représentation schématique des différents types d'estuaires selon Pritchard (1955). L'eau douce est représentée en blanc, l'eau de mer en gris foncé. La gamme de gris correspond au gradient de salinité.

Les deux courants hydrauliques opposés (eaux marines et continentales) forment dans l'estuaire des zones de circulation localisées. Les particules en suspension (matière en suspension = MES) se concentrent pour former le « bouchon vaseux » (Glangeaud, 1938), caractéristique des estuaires dominés par la marée (Wells, 1995). Généralement, il se localise dans la partie aval de l'estuaire à l'amont de l'intrusion saline (Potsma, 1961 ; Allen, 1974 ; Avoine, 1981 ; Castaing, 1981). Sa concentration varie fortement d'un estuaire à l'autre (Uncles et al., 2002a, 2002b). Les MES s'accumulent alors au niveau du point nodal sous trois formes : minérale, organique vivante et organique détritique, et peuvent amorcer un nouveau cycle (Castaing, 1981).

I.2.2 Caractéristiques biotiques

Les estuaires sont des milieux en constante évolution, très dynamiques et très complexe (partie I- 1.1). Les variations quotidiennes de salinité et de température induisent des grandes difficultés pour les organismes, c'est pourquoi peu d'entre eux sont réellement inféodés à ce milieu. La plupart des espèces trouvées dans les zones estuariennes ne sont que transitoires, et sont natifs soit de la mer soit de l'eau douce. De la même façon que la salinité, un « gradient » se crée au sein des estuaires, d'amont en aval. En effet, les espèces originaires d'eau douce vont petit à petit succomber au bénéfice des espèces euryhalines. En revanche, les espèces typiquement estuariennes vont occuper la zone saumâtre, là où le nombre d'espèces marines et d'eau douce est réduit, mais cette zone reste peu diversifiée (Remane, 1934 ; Telesh et al., 2014, Fig 2).

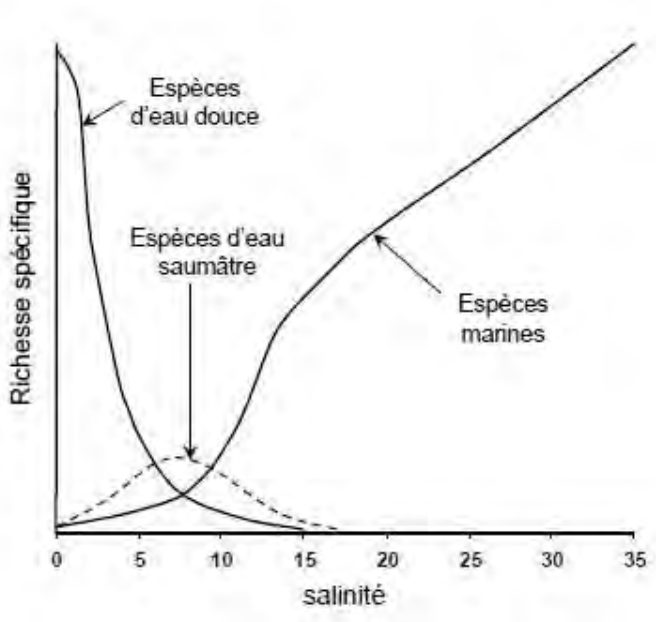


Figure 2 : Répartition de la richesse spécifique sur un gradient de salinité selon Remane (1934).

I.2.3 Le zooplancton au cœur des réseaux trophiques

I.2.3.1 Caractérisation du zooplancton

Zooplancton viens du grec *zoo* = « animal » et « πλαγκτός / *plagktós* » = errant, instable. Il constitue le principal compartiment animal aquatique en termes de

biomasse et de diversité à la fois dans les écosystèmes marins mais aussi dans les eaux douces (Mauchline, 1998). Longtemps il a été caractérisé par le fait de ne pas être capable de nager contre – courants. La définition passive du zooplancton a évolué depuis quelque temps, avec notament les travaux de Michaec et al. (2014) qui ont montré, par exemple qu'*E. affinis* peut nager juqu'à certaines limites dans un milieu turbulent. De nombreuses espèces viennent agrémenter ce groupe. Le plancton est subdivisé en cinq groupes selon leur taille qui sont (Dussart, 1965) :

- Mégazooplancton (20-200cm)
- Macrozooplancton (2-20cm)
- Mésozooplancton (0.2-2cm)
- Microzooplancton (20-200µm)
- Picozooplancton (0.2-2µm)

I.2.3.2 Le zooplancton estuarien

La composition mésozooplanctonique des zones marines et saumâtres dans les estuaires nord européens est essentiellement constituée de copépodes et de mysidacés (Mees et al., 1993 ; Sautour et Castel, 1995 ; Mouny et al., 2000 ; Mouny et Dauvin, 2002). L'intérêt des écosystèmes estuariens pour les espèces marines est lié à la présence de fortes populations zooplanctoniques, maillons clés permettant le transfert d'énergie du premier niveau trophique à la partie supérieure de la chaîne alimentaire, qui font des estuaires des zones de nurserie idéale pour le développement des larves et des juvéniles de crustacés et de poissons.

Les estuaires sont des lieux de passage d'importantes quantités de matière organique d'origine continentale et océanique transitant dans l'estuaire de l'amont vers l'aval ou de l'aval vers l'amont en fonction des rythmes tidaux et des fluctuations saisonnières des débits fluviaux. Afin d'assurer leurs besoins nutritionnels les organismes zooplanctoniques vont être soumis à ces contraintes, ainsi qu'aux contraintes d'abondance et de qualité du pool nutritif. Ces contraintes nutritionnelles entrainent des adaptations de la part du zooplancton. Il va donc développer des régimes alimentaires « omnivores opportunistes » en fonction de la disponibilité de la ressources (Heinle et al., 1977 ; Gasparini et Castel, 1997, Fockedey et Mees, 1999).

I.2.3.3 Les réseaux trophiques estuariens

D'après David et al. (2006), les réseaux trophiques estuariens sont déterminés par un fonctionnement basé sur des apports allochtones et par la multiplication des maillons trophiques entre la faible biomasse végétale détritique (biomasse macrovégétale, faible en qualité nutritive) et le zooplancton (Heinle et al., 1977 ; Hummel et al., 1988).

Lobry et al. (2008) ont montré l'utilisation optimale de la matière organique (MO) de faible qualité grâce à la l'enchaînement saisonnier d'espèces qui diversifient les flux trophiques et stabilise ainsi le système (fig. 3). Le zooplancton joue un rôle dans le transfert d'énergie (§ I.1.4.2), et, de plus, étant des proies pour d'autres maillons il joue aussi un rôle dans l'échange d'énergie à travers les poissons marins entre l'estuaire et la zone côtière.

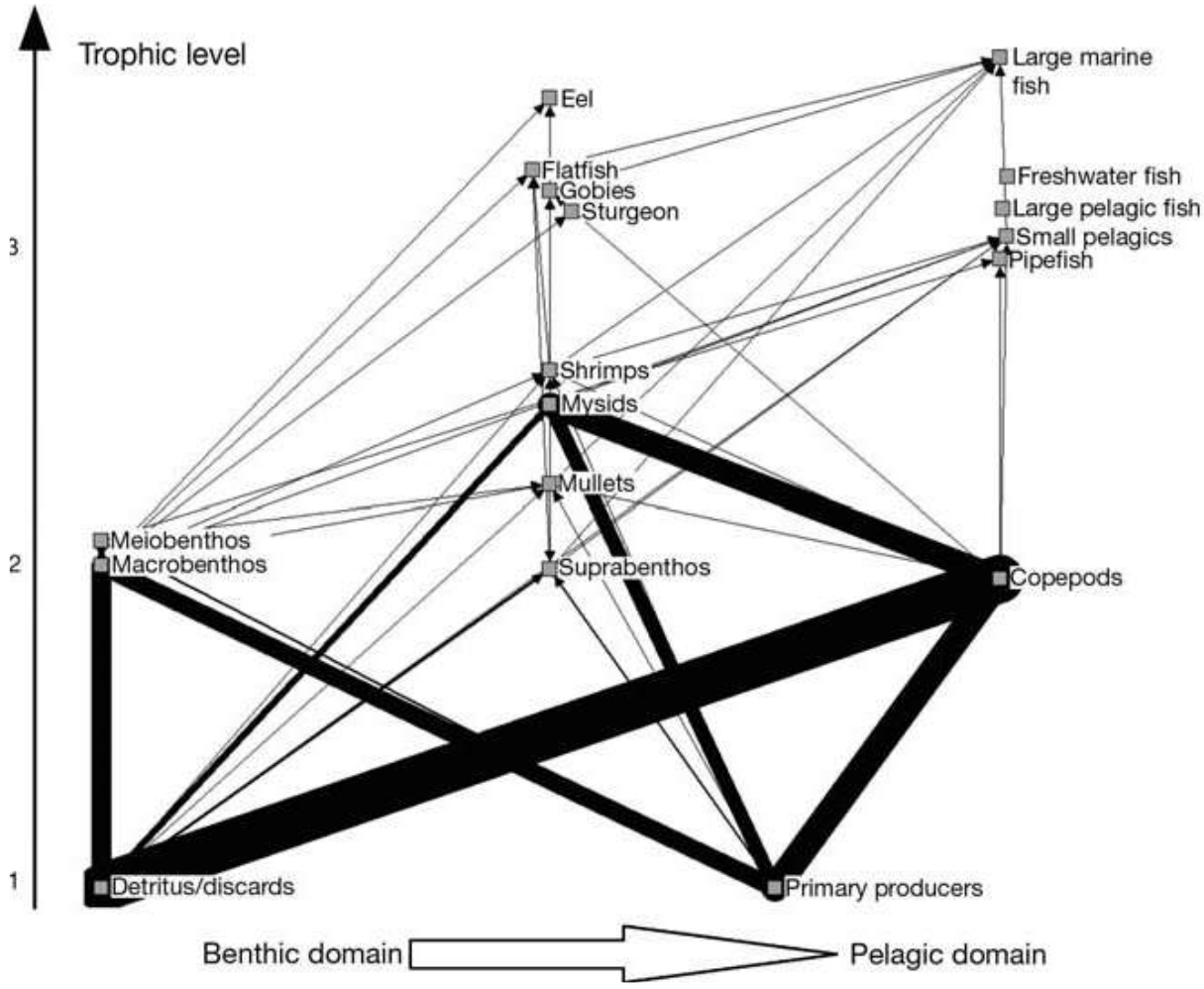


Figure 3 : Réseau trophique de l'estuaire de Gironde. Seuls des flux importants (flêches) et des compartiments (carrés) sont réprésentés (>90% de la valeur de débit total et 70 % des interactions totales). La largeur de chaque flêche est ajustée à la valeur de débit trophique (Lobry et al., 2008).

I.3 Cas d'étude : L'Escaut

I.3.1 Généralités

L'Escaut, Schelde en néerlandais et Scheldt en anglais, est un fleuve s'étendant sur 355km de long dont le bassin versant couvre une superficie de 21 863 km². Il prend sa source dans le nord de la France à Gouy-le-Câtelet (St Quentin). Avant de se jeter dans la mer l'Escaut traverse deux pays : la Belgique et les Pays-Bas (fig 4a). L'influence de la marée se fait ressentir jusqu'à 160 km de l'embouchure, soit au niveau de Gent, ou elle est arrêtée par des écluses.

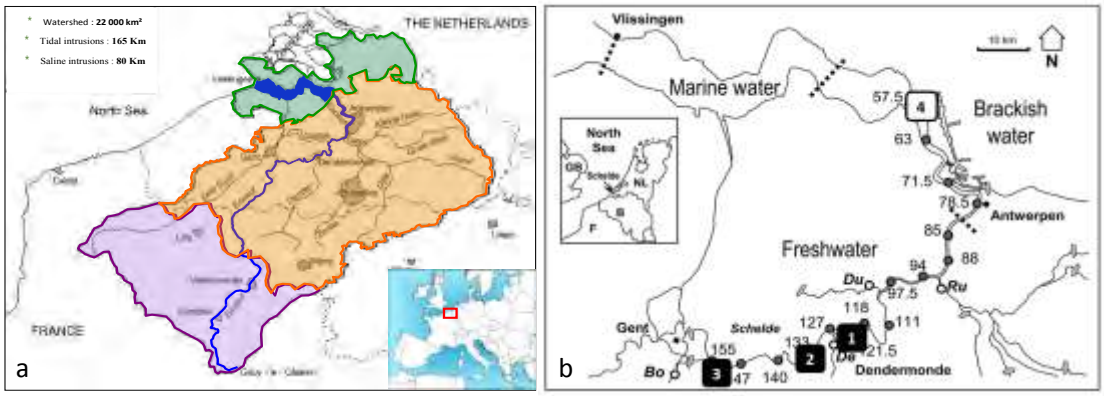


Figure 4 : a) Carte du bassin versant de l'Escaut, b) Map of the Scheldt estuary with OMES sampling stations, designated by their distance, in km, upstream from Vlissingen (mouth). Dotted lines indicate limits between marine water, brackish water and freshwater reaches. Antwerp station considered. Number 1 : Dendermonde, 2: Uitbergen and 3 : Melle.

I.3.2 La restauration

Au cours des années, un grand nombre de fonctionnalités écologiques ont connu des altérations voir même des pertes au sein des grands estuaires et ceci est essentiellement dû à un développement des activités anthropiques (e.g. Meire et al., 1992 ; Van Damme et al., 1997 ; Long et al., 2000 ;Verity 2002a, 2002b ; Baird et al., 2004). La restauration de ces écosystèmes irremplaçables est devenue essentielle (Van Damne at al., 2005 ; Istva´novics & Honti, 2012 ; Romero et al., 2016).

L'Escaut est l'un des estuaires le plus suivi au monde, les différents projets et/ou commissions ont permis de mettre en place des mesures restauratrices devant répondre plus efficacement à la dégradation de la qualité écologique de l'Escaut et de son bassin. En effet, depuis environ trente ans, menés par différents pays, de nombreux travaux de restauration ont eu lieu. Avec notamment, la mise en place du traitement des eaux usées sur le bassin versant de l'Escaut (Posel, 2007, Brion et al., 2016).

Au cours de l'histoire, des aménagements de l'Escaut en faveur de la navigation et la protection contre des inondations ont réduit fortement la taille des zones inondables et donc ont causé d'importantes modifications environnementales. En 2005, le plan SIGMA (plan d'aménagement de l'Escaut) est réactualisé afin de prendre en compte à la fois l'aspect sûreté pour la population mais aussi pour la nature. La restitution

d'espaces à la nature est donc engagée par le gouvernement flamand (Cox et al., 2006 ; Jacobs et al., 2008 ; Jacobs et al., 2009).

Le programme scientifique OMES (Onderzoek Milieu-effecten Sigma-plan = « recherche sur les conséquences du plan Sigma ») a été créé en parallèle afin de pallier au manque de connaissances sur la partie supérieure de l'estuaire situé entre Gand et Anvers (« Zeeschelde »). Ce programme commandé par le gouvernement Flamand permet depuis novembre 1995 un suivi conséquent et multidisciplinaire de l'écologie de la partie d'eau douce estuarienne de l'Escaut. A l'heure actuelle, on constate que la restauration du milieu a entrainé une amélioration notable de la qualité de l'eau. Parmi les divers organismes suivis dans le cadre d'OMES, on retrouve le zooplancton.

I.3.3 Contexte physico-chimique

I.3.3.1 Régime hydraulique

L'Escaut est de type D (Fig. 1), c'est-à-dire qu'on retrouvera des courants de marées souvent plus importants que les débits fluviaux. En effet, les marées se propagent de Vlissingen jusqu'à la ville de Gand avec des hauteurs respectives de 4 et 2 m et y sont arrêtées par les barrages-écluses de Gentbrugge (Fig. 4b). La figure 5 révèle un faible débit depuis 2004 et notamment dans la partie d'eau douce. L'influence de la marée ainsi que les faibles débits vont induire un temps de résidence variable mais élevé, estimé à 2 ou 3 mois pour la totalité de l'estuaire (Soetaert and Herman, 1995).

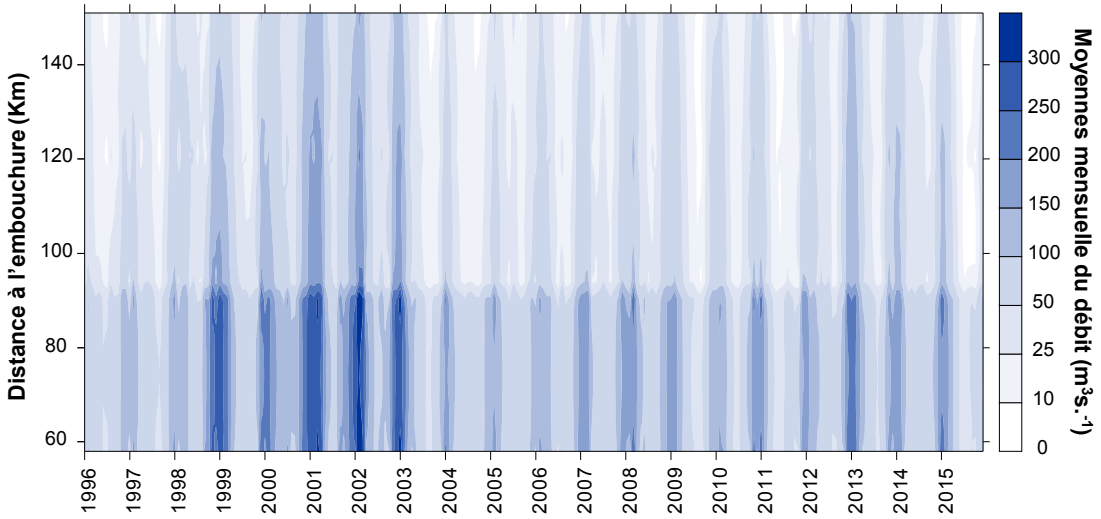


Figure 5 : Moyenne mensuelle des débits dans l'estuiare de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.

I.3.3.2 Salinité

Comme vue précédemment, l'Escaut est de type D, il y a donc peu de stratification verticale en termes de salinité. En revanche il y a un fort gradient de salinité au niveau horizontal (Van Eck et al., 1997 ; Baeyens et al., 1998). En fonction des apports hydrologiques issus des débits il y aura des variations de salinité, c'est pourquoi selon la saison au niveau d'Anvers on retrouve de l'eau plus ou moins salée. L'Escaut va donc être subdivisé en trois zones : une zone polyhaline (salinité >10), une zone saumâtre (0.5<salinité< 10) et une zone d'eau douce (salinité <0.5) (fig. 7).

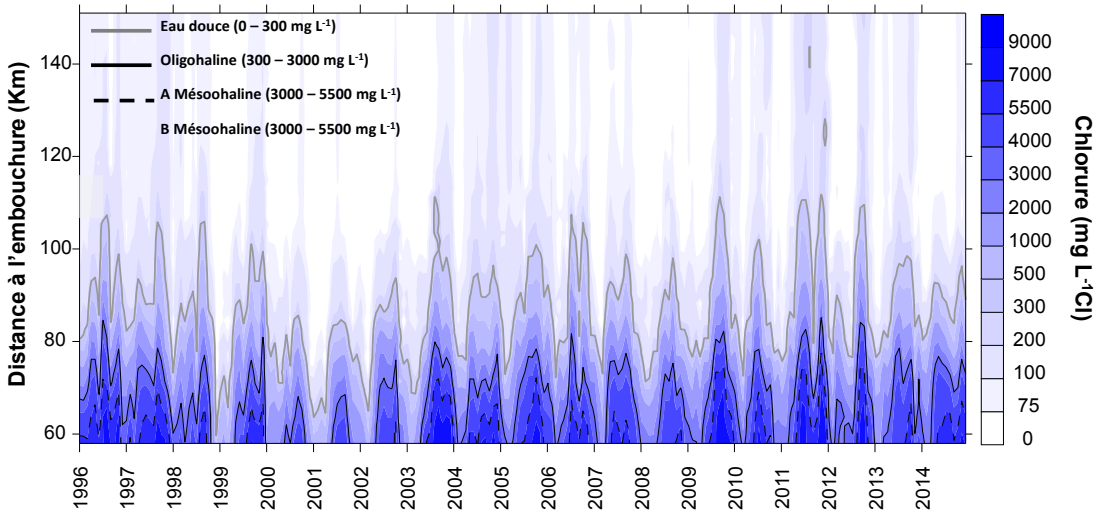


Figure 6 : Chloronité dans l'estuaire de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.

I.3.3.3 Concentration en oxygène

L'amélioration de la qualité de l'eau de l'estuaire se révèle notamment par l'augmentation de la concentration en oxygène dissous du milieu. Durant des années la concentration en oxygène a été très faible (<0.08 mg L^{-1}, fig., 7) sur tout le tronçon Grens – Melle. Ensuite, seule une zone de déficit en oxygène aux alentours d'Anvers (km 78) a persisté, résultant des rejets d'eaux usées. Durant la dernière décennie la concentration en oxygène dissous est relativement stable et égale entre l'eau saumâtre et l'eau douce.

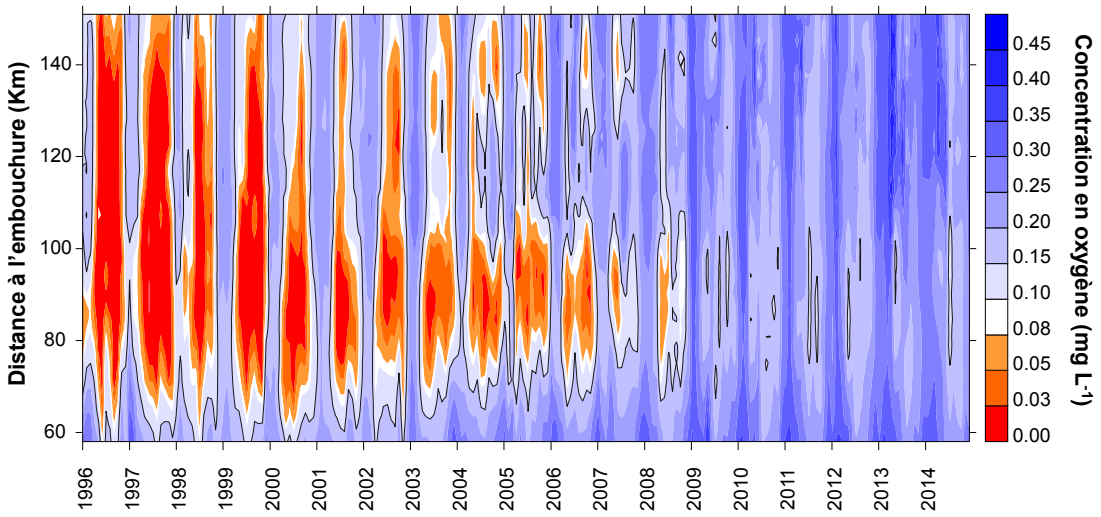


Figure 7 : Concentration en oxygène dans l'estuaire de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.

I.3.3.4 Concentration en ammonium

L'azote est un élément essentiel de la matière vivante, pouvant se présenter sous plusieurs formes dans les milieux aquatiques. Ce paragraphe, fait référence à l'azote ammoniacal ($N\text{-}NH_4$), qui est un élément présent dans les milieux aquatiques riches en matières organiques en décomposition. Au-delà d'une concentration de 2 mg L^{-1} de $N\text{-}NH_4$ constitue un élément toxique pour le milieu. La figure 8, montre une concentration supérieure à 2 mg L^{-1} dans l'Escaut jusqu'en 2006, puis une forte diminution pour atteindre des valeurs en dessous de 2 mg L^{-1} ces dernières années.

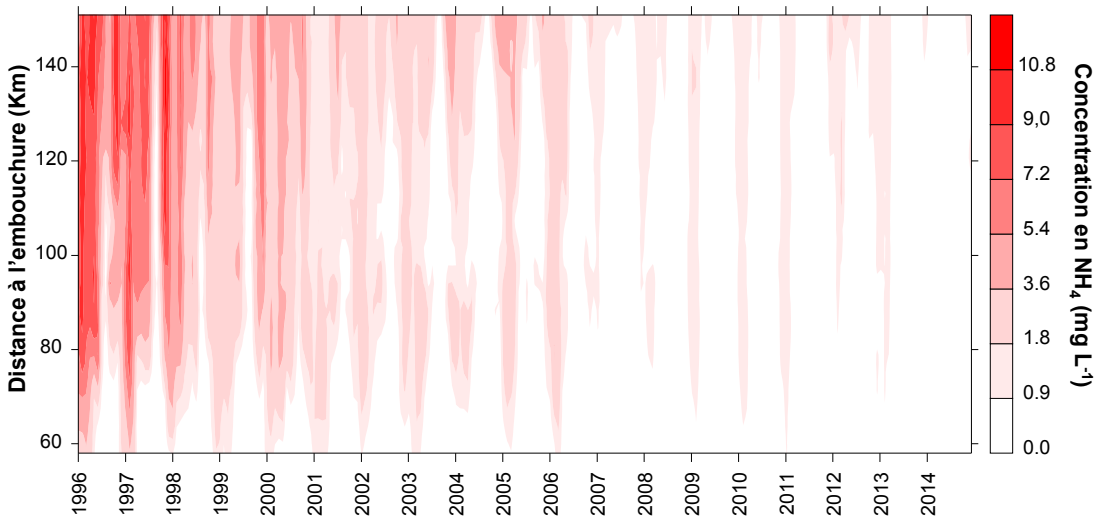


Figure 8 : Concentration en NH_4 dans l'estuaire de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.

I.3.3.5 Matière en suspension

Les matières en suspension totale (MES) sont l'un des facteurs importants du fonctionnement du système estuarien. Dans l'Escaut (fig 9), la majeure partie de la MES est détritique. La dégradation bactérienne de la matière organique contenue dans les MES entraine des déficits de la concentration en oxygène dissous (Van Damme et al., 2005). La matière détritique, semble également être une source de nourriture, notamment pour le zooplancton (Heinle et al., 1977 ; Harris et al., 1977 ; Castel & Feurtet, 1989).

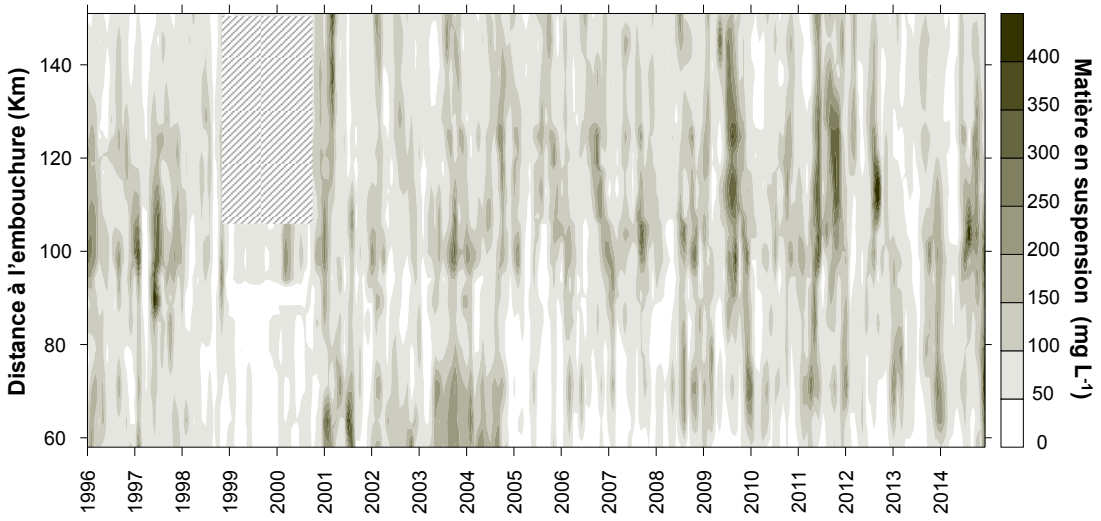


Figure 9 : Concentration de la MES dans l'estuaire de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.

I.3.3.6 Production primaire et biomasse phytoplanctonique

Les travaux de Cox et al. (2009), montrent un changement de milieu d'hypereutrophe à eutrophe dans l'Escaut en quatre étapes. L'étape 1 représente l'état hypereutrophié, caractérisé par des concentrations d'ammonium élevées, l'hypoxie ou l'anoxie du milieu ainsi que l'inhibition de la croissance des algues. La phase de transition (étape2), ou le milieu est passé de l'état hypereutrophié à l'état d'eutrophisation classique. L'étape 3 montre l'état eutrophisé avec les efflorescences algales intenses. La continuation des apports en nutriments et l'activité de broutâge du zooplancton peuvent amaner à la dernière étape, avec une réduction des efflorescences algales intenses.

Depuis que les concentrations en oxygène se sont améliorées, la production primaire du phytoplancton a pu reprendre. La concentration en Chl *a* en eau douce a augmenté (fig. 10).

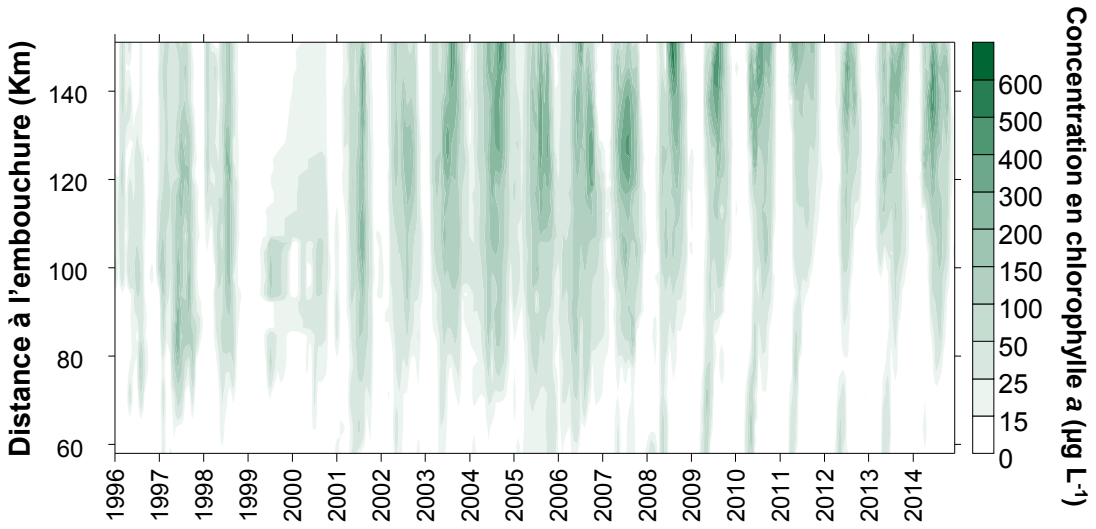


Figure 10 : Concentration de la Chl *a* dans l'estuaire de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.

Les comptages phytoplanctoniques, réalisés à l'université de Gand (Research Group Protistology aand Aquatic Ecology ; PAE), montrent un changement au sein des communautés de phytoplancton. Tout d'abord, il y a une augmentation de la biomasse des chlorophytes depuis 2009, puis un changement au sein de la communauté des diatomées, avec une inversion de dominance entre petites et grandes diatomées. Après une dominance de *Cyclotella* spp. (1996-2002), l'espèce *Actinocyclus normannii* était dominante en 2003-2008 mais depuis 2009 c'est *Cyclotella* spp. qui redevient dominante.

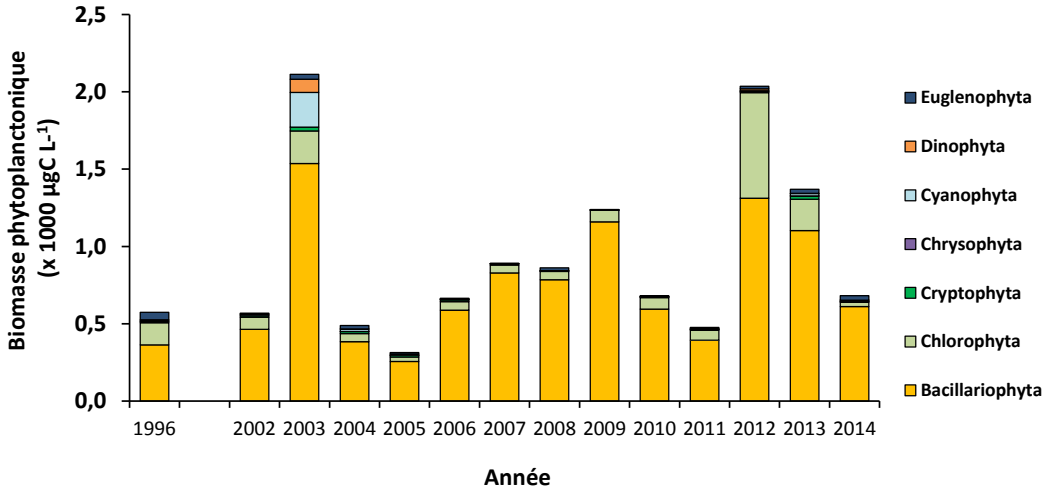


Figure 11 : Biomasse phytoplanctionique de la zone d'eau douce de l'estuaire de l'Escaut en fonction des années. Données issues des travaux de l'université de Gand, PAE.

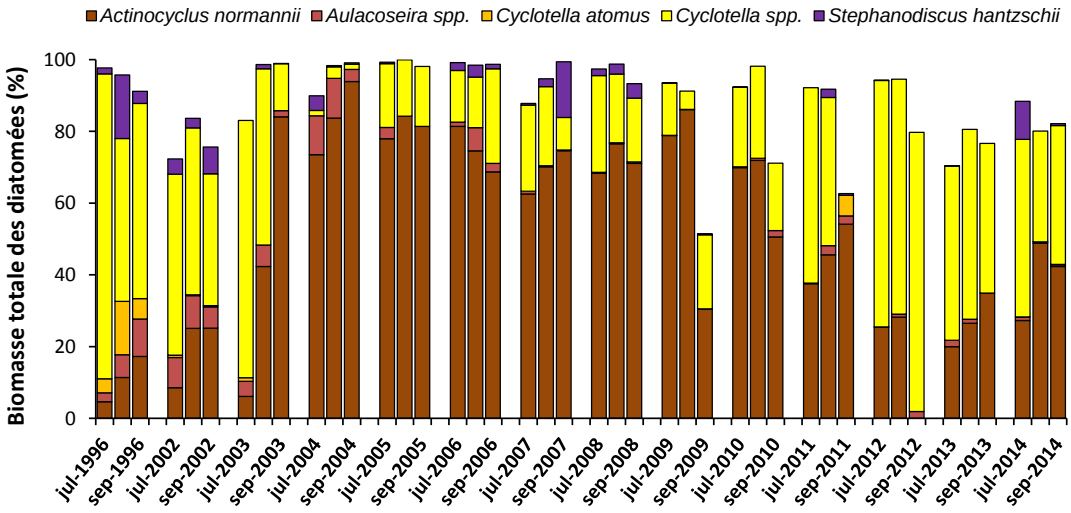


Figure 12 : Contribution à la biomasse phytoplanctonique totale des 5 taxons dominants de diatomées de la zone d'eau douce de l'estuaire de l'Escaut en fonction des années. Données issues des travaux de l'université de Gand, PAE.

I.3.4 Le zooplancton de l'Escaut

I.3.4.1 Généralités

Rendant disponible la matière organique issue des producteurs primaires pour les consommateurs secondaires, le zooplancton occupe une position clé au sein des réseaux trophique pélagiques (Sautour et Castel, 1995 ; Tackx et al., 2003; Maes et al., 2005). Wilson (1994) et Appeltans et al. (2003) vont jusqu'à leur allouer un rôle de bioindicateur. Au cours d'une succession de projets, la dynamique zooplanctonique de l'Escaut a pu être suivie. Les études ont débuté à la fin des années 60 avec les travaux de De Pauw (1975), qui a quantifié le zooplancton pendant la période 1967-1969 de Vlissingen à Gand. Durant cette période, la communauté zooplanctonique du tronçon d'eau douce (la Zeeschelde) était dominée par des rotifères, des cyclopoides et des cladocères. Le copépode calanoide *Eurytemora affinis* était une espèce importante dans l'Escaut, observée de l'embouchure jusqu' à Anvers, avec un maximum au printemps dans la zone d'eau saumâtre. Après un longue intérruption dans la recherche sur le zooplancton de l'Escaut, Soetaert & Van Rijswijk (1993) ont quantifié les communautés zooplanctoniques entre 1989 et 1991 de Vlissingen à Anvers. Ils observent essentiellement la même composition de la communauté en eau saline et saumâtre que rapportée par De Pauw (1975), mais la zone en amont d'Anvers est caractérisée par une très faible densité de zooplancton. Les travaux qui ont suivi se sont concentrés plus particulièrement sur l'eau saumâtre de l'Escaut. La dominance d'*E. affinis* au printemps dans cette zone est suivie par un déclin de la population d'*E. affinis* pour laisser place au calanoïde *Acartia tonsa* en été (De Pauw, 1975 ; Soetaert et Van Rijswijk, 1993 ; Sautour et al., 1995 ; Tackx et al., 2004 ; Azemar et al., 2007). Avec les travaux de Mialet et al. (2010), on constate une la population d'*E. affinis* a drastiquement étendue sa position dans l'Escaut après 2007. La population d'*E. affinis* s'est égalemente développé dans le tronçon d'eau douce et y est maintenant dominante.

I.3.4.2 *E. affinis*

E. affinis (Poppe, 1880), copépode calanoïde, fait partie des espèces l'une des plus étudiées. Pouvant s'acclimater rapidement à des fluctuations de salinité (Roddie et al., 1984, Devreker et al., 2004, 2009 ; Michalec et al., 2010) elle est présente dans un très grand nombre d'estuaires notamment dans l'hémisphère nord, et y est souvent l'une des bases essentielles de leurs chaînes trophiques (Fockedey & Mees, 1999 ; Winkler & Greve, 2004).

Cette espèce est typiquement estuarienne et, est située dans zones oligo-mésohaline à des salinités comprises entre 0 et 18 (Vaupel-Kleine & Weber, 1975 ; Mouny et al., 1998 ; Lawrence et al., 2004).

Différentes études ont révélé la présence de cette espèce dans les eaux douces estuariennes et ce en Amérique du Nord, en Europe ou encore en Asie (Lee, 1999 ; David et al., 2005 ; Mialet et al., 2010). De plus, cette espèce souvent retrouvée dans des zones turbides et ayant la capacité à sélectionner sa ressource alimentaire (Sautour and Castel, 1995 ; Billones et al., 1999 ; Gasparini et al., 1999 ; Tackx et al., 2003) dans un milieu essentiellement détritique, présente un grand intérêt ecosystémique. *Eurytemora affinis* domine fortement la communauté mésozooplanctonique de l'estuaire de l'Escaut puisque celui-ci peut représenter, en termes de densité, jusqu'à 70% du mésozooplancton (Chambord et al., accepté). *E. affinis* est par conséquence un maillon essentiel de l'écosystème estuarien de l'Escaut.

I.4 Problématique et objectifs de la thèse

I.4.1 Contexte

Le projet OMES a pour but d'aboutir à un modèle d'écosystème qui aidera les décideurs à gérer ce système estuarien (Meire et al., 1997). De façon générale, ce programme de recherche permet l'acquisition de données sur la répartition et le rôle fonctionnel de divers compartiments. Cette acquisition de données multidisciplinaires, comprenant des données biotiques mais aussi des données abiotiques, permet de mettre en avant le suivi suite à la restauration du milieu.

Inscrit dans le projet OMES, le travail réalisé par cette thèse s'inscrit dans la continuité des études déjà démarrées concernant la dynamique de la communauté

mésozooplanctonique de l'estuaire. Ce travail a considéré de nouveaux éléments sur les communautés mésozooplanctoniques dans la partie d'eau douce de l'estuaire, d'une part par une étude des communautés, via le monitoring d'OMES et d'autre part par des expériences *in semi-situ* sur des processus fonctionnels tels que la tolérance à des basses concentrations en oxygène et les relations proie-prédateur.

I.4.2 Objectifs

Afin de répondre aux attentes de ce projet, l'ensemble du travail de thèse s'est articulé autour de quatre principaux objectifs. Les objectifs 1 et 2 sont détaillés dans le chapitre 2.

Objectif 1 : Cet objectif vise à **vérifier les perspectives issues des travaux de Mialet et al**. (2010), sur la répartition spatiale du mésozooplancton dans l'amont de l'estuaire. Notament, la durabilité dans le temps de la population d'*E. affinis* installée en eau douce.

Objectif 2 : Une fois la distribution spatiale des communautés mise en avant, cette étude s'est attachée à **déterminer** pour chaque grand taxon zooplanctonique **les facteurs favorisant leur développement** respectif.

Objectif 3 : Suite à la restauration du milieu, la concentration en oxygène a considérablement augmentée. Ce facteur est passé de limitant à optimal pour le développement de certaines espèces. Afin d'estimer **l'impact des faibles concentrations en oxygène** sur les différents taxa de zooplancton, des expériences *in semi-situ* ont été réalisées, les résultats font l'objet du chapitre 3.

Objectif 4 : Le chapitre 4 de cette étude concerne le fonctionnement trophique de l'amont de l'Escaut, via le mésozooplancton et plus particulièrement la **sélectivité de l'espèce dominante *E. affinis***. Et enfin, nous nous sommes intéressés à la caractérisation de **l'impact du broutage** de cette espèce (représentant 70% du mésozooplancton dans la partie eau douce de l'Escaut) **sur le phytoplancton**.

I.5 Références bibliographiques

A --

Appeltans, W., Hannouti, A., van Damme, S., Soetaert, K., Vanthomme, R. and Tackx, M. (2003). Zooplankton in the Schelde estuary (Belgium/The Netherlands). The distribution of *Eurytemora affinis*: effect of oxygen? Journal of Plankton Research, 25(11), 1441-1445.

Allen, G. P., Bonnefille, R., Courtois, G., & Migniot, C. (1974). Processus de sédimentation des vases dans l'estuaire de la Gironde Contribution d'un traceur radioactif pour l'étude du déplacement des vases. La Houille Blanche, (1-2), 129-136.

Ashley, R., & Cashman, A. (2006). The impacts of change on the long-term future demand for water sector infrastructure. Infrastructure to 2030: Telecom, Land Transport, Water and Electricity, 241-349.

Azémar, F. (2007). L'écologie du zooplancton de l'estuaire de l'Escaut (Belgique). (Thèse de doctorat, Université Paul Sabatier Toulouse III), pp. 186.

B --

Baeyens, W., Van Eck, B., Lambert, C., Wollast, R. and Goeyens, L. (1998). General description of the Scheldt estuary. In Trace Metals in the Westerschelde Estuary: A Case-Study of a Polluted, Partially Anoxic Estuary (pp. 1-14). Springer NetherlandsBan et al., 1994.

Baird, D., Christian, R. R., Peterson, C. H., & Johnson, G. A. (2004). Consequences of hypoxia on estuarine ecosystem function: Energy diversion from consumers to microbes. Ecological Applications, 14(3), 805-822.

Billones, R. G., Tackx, M. L. M., Flachier, A. T., Zhu, L., & Daro, M. H. (1999). Image analysis as a tool for measuring particulate matter concentrations and gut content, body size, and clearance rates of estuarine copepods: validation and application. Journal of Marine Systems, 22(2), 179-194.

Brion, N., Verbanck, M. A., Bauwens, W., Elskens, M., Chen, M., Servais, P., 2015. Assessing the impacts of wastewater treatment implementation on the water quality of a small urban river over the past 40 years. Environmental Science and Pollution Research, 22(16), 12720-12736.

C --

Castel, J., & Feurtet, A. G. N. È. S. (1989). Dynamics of the copepod *Eurytemora affinis* hirundoides in the Gironde Estuary: Origin and fate of its production. Sci. Mar., 53(2), 577-584.

Castaing, P. (1981). Le transfert à l'océan des suspensions estuariennes: cas de la Gironde (Vol. 12). Original typescript.

Costanza, R., Darge, R., Degroot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., Oneill, R., Paruelo, J., Raskin, R., Sutton, P. and Vandenbelt, V. 1997. The value of the world's ecosystem services and natural capital. Nature 387, 253-260.

Cox, D. J., Punja, M., Powers, K., Merkel, R. L., Burket, R., Moore, M., & Kovatchev, B. (2006). Manual Transmission Enhances Attention and Driving

Performance of ADHD Adolescent Males Pilot Study. Journal of attention disorders, 10(2), 212-216.

Cox, T. J. S., Maris, T., Soetaert, K., Conley, D. J., Van Damme, S., Meire, P. and Struyf, E. (2009). A macro-tidal freshwater ecosystem recovering from hypereutrophication: the Schelde case study. Biogeosciences, 6, 2935-2948.

D --

David, V., Sautour, B., Chardy, P., Leconte, M., 2005. Long-term changes of the zooplankton variability in a turbid environment: the Gironde estuary (France). Estuarine, Coastal and Shelf Science, 64(2), 171-184.

David, V., Sautour, B., Galois, R., & Chardy, P. (2006). The paradox high zooplankton biomass–low vegetal particulate organic matter in high turbidity zones: What way for energy transfer? Journal of Experimental Marine Biology and Ecology, 333(2), 202-218.

De Pauw, N., 1975. Bijdrage tot de kennis van milieu en plankton in het Westerschelde estuarium. PhD, thesis University of Ghent (Belgium). I, Dutch.

Devreker, D., Souissi, S., & Seuront, L. (2004). Development and mortality of the first naupliar stages of *Eurytemora affinis* (Copepoda, Calanoida) under different conditions of salinity and temperature. Journal of Experimental Marine Biology and Ecology, 303(1), 31-46.

Devreker, D., Souissi, S., Winkler, G., Forget-Leray, J., Leboulenger, F., 2009. Effects of salinity, temperature and individual variability on the reproduction of *Eurytemora affinis* (Copepoda; Calanoida) from the Seine estuary: a laboratory study. Journal of Experimental Marine Biology and Ecology, 368(2), 113-123.

Dionne, J. C. (1963). Vers une définition plus adéquate de l'estuaire du Saint-Laurent. *Z. Geomorph*, *7*, 36-44.

Dussart, B. H. (1965). Les différentes catégories de plancton. Hydrobiologia, 26(1), 72-74.

Dyer, K. (1986). Coastal and estuarine sediment dynamics. *Chichester: Wiley*.

Dyer, K.R. (1997). Estuaires: A physical introduction: Wiley, London eds, 140 p.

E, F --

Elliott, M., & Ducrotoy, J. P. (1991). *Estuaries and coasts: spatial and temporal intercomparisons* (Vol. 19). Olsen & Olsen.

Elliott, M., & McLusky, D. S. (2002). The need for definitions in understanding estuaries. *Estuarine, Coastal and Shelf Science*, *55*(6), 815-827.

Fockedey, N., & Mees, J. (1999). Feeding of the hyperbenthic mysid *Neomysis integer* in the maximum turbidity zone of the Elbe, Westerschelde and Gironde estuaries. *Journal of Marine Systems*, *22*(2), 207-228.

G, H, I--

Gasparini, S., & Castel, J. (1997). Autotrophic and heterotrophic nanoplankton in the diet of the estuarine copepods *Eurytemora affinis* and *Acartia bifilosa*. Journal of Plankton Research, 19(7), 877-890.

Glangeaud, L. (1938). Transport et sédimentation dans l'estuaire et à l'embouchure de la Gironde. Caractères pétrographiques des formations fluviatiles, saumâtres, littorales et néritiques. *Bulletin de la Societe Geologique de France, Paris*, *7*(5), 599-630.

Heinle, D. R., Harris, R. P., Ustach, J. F., & Flemer, D. A. (1977). Detritus as food for estuarine copepods. Marine Biology, 40(4), 341-353.

Hummel, H., Moerland, G., & Bakker, C. (1988). The concomitant existence of a typical coastal and a detritus food chain in the Westerschelde estuary. Hydrobiological Bulletin, 22(1), 35-41.

Istvánovics, V., & Honti, M. (2012). Efficiency of nutrient management in controlling eutrophication of running waters in the Middle Danube Basin. Hydrobiologia, 686(1), 55-71.

J, L --

Jacobs, S., Struyf, E., Maris, T., & Meire, P. (2008). Spatiotemporal aspects of silica buffering in restored tidal marshes. Estuarine, Coastal and Shelf Science, 80(1), 42-52.

Jacobs, S., Beauchard, O., Struyf, E., Cox, T., Maris, T., & Meire, P. (2009). Restoration of tidal freshwater vegetation using controlled reduced tide (CRT) along the Schelde Estuary (Belgium). Estuarine, Coastal and Shelf Science, 85(3), 368-376.

Lawrence, D., Valiela, I., & Tomasky, G. (2004). Estuarine calanoid copepod abundance in relation to season, salinity, and land-derived nitrogen loading, Waquoit Bay, MA. Estuarine, Coastal and Shelf Science, 61(3), 547-557.

Lee, C.E., Bell, M. A., 1999. Causes and consequences of recent freshwater invasions by saltwater animals, Trends in Ecology & Evolution 14(1), 284-288.

Lobry, J., David, V., Pasquaud, S., Lepage, M., Sautour, B. & Rochard, E. (2008). Diversity and stability of an estuarine trophic network. Marine Ecology Progress Series, 358, 13-25.

Long, E. R. (2000). Degraded sediment quality in US estuaries: a review of magnitude and ecological implications. Ecological applications, 10(2), 338-349.

M --

Mauchline, J. (1998). The biology of plankton copepods. *Adv mar biol*, *33*.

Mees, J., Dewicke, A., & Hamerlynck, O. (1993). Seasonal composition and spatial distribution of hyperbenthic communities along estuarine gradients in the Westerschelde. Netherland Journal of Aquatic Ecology, *27*(2-4), 359-376.

Meire, P., Rossaert, G., & De Regge, N. (1992). Het Schelde estuarium: ecologische beschrijving en visie op de toekomst. Instituut voor Natuurbehoud.

Meire, P., Starink, M., & Hoffman, H. (1997). Integratie van ecologie en waterbouwkunde in de Zeeschelde: aanleiding tot en situering van het onderzoek milieu-effecten sigmaplan (OMES). Water: Tijdschrift over Waterproblematiek, (95).

Mialet, B., Azémar, F., Maris, T., Sossou, C., Ruiz, P., Lionard, M., Van Damme, S., Lecerf, A., Muylaert, K., Toumi, N., Tackx, M. (2010). Spatial spring distribution of the copepod *Eurytemora affinis* (Copepoda, Calanoida) in a restoring estuary, the Scheldt (Belgium), Estuarine, Coastal and Shelf Science, 88, 116-124.

Michalec, F. G., Souissi, S., Dur, G., Mahjoub, M. S., Schmitt, F. G., & Hwang, J. S. (2010). Differences in behavioral responses of *Eurytemora affinis* (Copepoda, Calanoida) reproductive stages to salinity variations. Journal of Plankton Research, fbq006.

Michalec, F. G., Souissi, S. & Holzner, M. (2015). Turbulence triggers vigorous swimming but hinders motion strategy in planktonic copepods. Journal of The Royal Society Interface, 12(106), 20150158.

Mouny, P., & Dauvin, J. C. (1996). Les communautés mesozooplanctoniques et suprabenthiques de l'estuaire de la Seine. Rapport d'activité du Programme ScientifiqueSeine-Aval'. Rapport, 62-98.

Mouny, P., Dauvin, J. C., Bessineton, C., Elkaim, B., & Simon, S. (1998). Biological components from the Seine estuary: first results. Hydrobiologia, 373, 333-347.

Mouny, P., Dauvin, J. C., & Zouhiri, S. (2000). Benthic boundary layer fauna from the Seine estuary (eastern English Channel, France): spatial distribution and seasonal changes. Journal of the Marine Biological Association of the UK, 80(06), 959-968.

Mouny, P., & Dauvin, J. C. (2002). Environmental control of mesozooplankton community structure in the Seine estuary (English Channel). Oceanologica Acta, 25(1), 13-22.

P – –

Postma, H., 1961. Transport and accumulation of suspended matter in the Dutch Wadden Sea Neth. J. Sea Res., 1 (1961), pp. 148–190.

Perillo, G. M. (1995). Definitions and geomorphologic classifications of estuaries. Geomorphology and sedimentology of estuaries. Developments in Sedimentology, 53, 17-47.

Pritchard, D. W., 1955. Estuarine circulation patterns. Proceedings of the American Society of Civil Engeneers 81, 1-11.

Pritchard, D. W., 1967. What is an estuary: physical view-point. American Association for the advancement of Science Publication, Washington, DC (USA), 757 pp.

Potter, I. C., Chuwen, B. M., Hoeksema, S. D., & Elliott, M. (2010). The concept of an estuary: a definition that incorporates systems which can become closed to the ocean and hypersaline. Estuarine, Coastal and Shelf Science, 87(3), 497-500.

Posel, S., 2007. Les eaux usées bruxelloises totalement épurées. Escaut sans Frontières Info, 12.

R --

Remane, A. (1934). Die Brackwasserfauna. Verhandlungen der Deutschen Zoologischen Gesellschaft, 36, 34-74.

Reid, G. K. (1961). Ecology of inland waters and estuaries.

Roddie, B. D., Leakey, R. J. G., & Berry, A. J. (1984). Salinity-temperature tolerance and osmoregulation in *Eurytemora affinis* (Poppe) (Copepoda: Calanoida) in relation to its distribution in the zooplankton of the upper reaches of the Forth estuary. Journal of Experimental Marine Biology and Ecology, 79(2), 191-211.

Romero, E., Le Gendre, R., Garnier, J., Billen, G., Fisson, C., Silvestre, M., & Riou, P. (2016). Long-term water quality in the lower Seine: Lessons learned over 4 decades of monitoring. Environmental Science & Policy, 58, 141-154.

S --

Sautour, B. and Castel, J. (1995). Comparative spring distribution of zooplankton in three macrotidal European estuaries. Hydrobiologia, 311(1-3), 139-151.

Soetaert, K., & Herman, P. M. (1995). Nitrogen dynamics in the Westerschelde estuary (SW Netherlands) estimated by means of the ecosystem model MOSES. Hydrobiologia, 311(1-3), 225-246.

Soetaert, K. and Van Rijswijk, P. (1993). Spatial and temporal patterns of the zooplankton in the Westerschelde estuary. Marine Ecology Progress Series. 97(1), 47-59.

Struyf, E., Van Damme, S., Gribsholt, B. & Meire, P., 2005. Freshwater marshes as dissolved silica recyclers in an estuarine environment (Schelde estuary, Belgium). Hydrobiologia 540, 69-77.

T, U--

Tackx, M. L. M., Herman, P. J. M., Gasparini, S., Irigoien, X., Billiones, R., & Daro, M. H. (2003). Selective feeding of *Eurytemora affinis* (Copepoda, Calanoida) in temperate estuaries: model and field observations. Estuarine, Coastal and Shelf Science, 56(2), 305-311.

Tackx, M. L., De Pauw, N., Van Mieghem, R., Azémar, F., Hannouti, A., Van Damme, S. and Meire, P. (2004). Zooplankton in the Schelde estuary, Belgium and The Netherlands. Spatial and temporal patterns. Journal of Plankton Research, 26(2), 133-141.

Telesh, I. V., Schubert, H., & Skarlato, S. O. (2010). Revisiting Remane's concept: evidence for high plankton diversity and a protistan species maximum in the horohalinicum of the Baltic Sea. Marine Ecology Progress Series, 421, 1-11.

Uncles, R.J. (2002a). Estuarine physical processes research: some recent studies and progress. Estuarine, Coastal and Shelf Science, 55, p. 829- 856.

Uncles, R.J., Stephens, J.A., Smith, R.E. (2002b). The dependence of estuarine turbidity on tidal intrus. Continental Shelf Research, 22, p. 1835-1856.

V, W, Z --

Van Damme, S., P. Meire, H. Maeckelberghe, M. Verdievel, L. Bourgoing, E. Taverniers, T. Ysebaert & G. Wattel, 1995. De waterkwaliteit van de Zeeschelde: evolutie in de voorbije dertig jaar. Water 85: 244–256.

Verity, P.G., 2002a. A decade of change in the Skidaway river estuary. I. Hydrography and nutrients. Estuaries 25(5), 944-960.

Verity, P.G., 2002b. A decade of change in the Skidaway river estuary. II. Particulate organic carbon, nitrogen and chlorophyll *a*. Estuaries 25(5), 961-975.

Von Vaupel-Klein, J. C., & Weber, R. E. (1975). Distribution of *Eurytemora affinis* (Copepoda: Calanoida) in relation to salinity: field and laboratory observations. Netherlands Journal of Sea Research, 9(3-4), 297-310.

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Reidy-Liermann, C. & Davies, P. M. (2010). Global threats to human water security and river biodiversity. Nature, 467(7315), 555-561.

Wells, J. T. (1995). Tide-dominated estuaries and tidal rivers. Developments in Sedimentology, 53, 179-205.

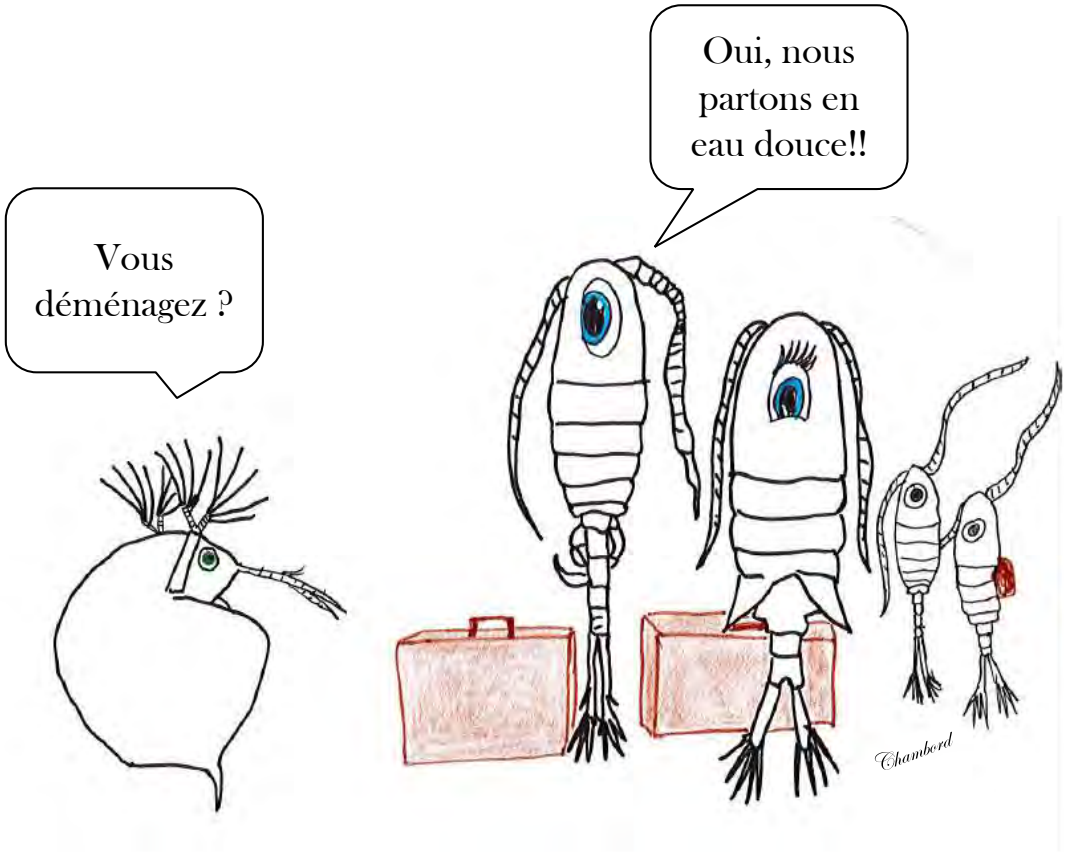
Wilson, J. G. (1994). The role of bioindicators in estuarine management. Estuaries, 17(1), 94-101.

Winkler, G., & Greve, W. (2004). Trophodynamics of two interacting species of estuarine mysids, Praunus flexuosus and Neomysis integer, and their predation on the calanoid copepod *Eurytemora affinis*. Journal of experimental marine Biology and Ecology, 308(1), 127-146.

Zwolsman, J. J., Van Eck, B. T., & Van Der Weijden, C. H. (1997). Geochemistry of dissolved trace metals (cadmium, copper, zinc) in the Scheldt estuary, southwestern Netherlands: impact of seasonal variability. Geochimica et Cosmochimica Acta, 61(8), 1635-1652.

II. CHAPITRE 2 :

Les affinités du mésozooplancton dans l'eau douce de l'estuaire de l'Escaut



II.1 Résumé

II.1.1 Contexte et objectifs

La qualité de l'eau de l'estuaire de l'Escaut s'est considérablement améliorée depuis les années 1990, en particulier en amont. Sachant sa position clé au sein des réseaux trophiques (Tackx et al., 2003 ; Maes et al., 2005b) ainsi que son rôle potentiel de bioindicateur (Appeltans et al., 2003), le zooplancton de l'estuaire est étudié depuis 1996 dans le cadre du programme OMES. Ces études ont permis de révéler des changements dans les communautés zooplanctoniques. En effet, le copépode *Eurytemora affinis*, généralement abondant dans les eaux saumâtres et quasi-absents de l'eau douce avant 2007, s'est depuis sensiblement développé dans les eaux douces (Mialet et al., 2011). Il représente désormais 70 % de l'abondance mésozooplanctonique de l'eau douce. Simultanément, l'abondance des copépodes cyclopoïdes a considérablement diminué dans la zone d'eau douce, tandis que l'abondance des cladocères n'a pas changé.

Objectifs de cette étude :

1) L'évolution de la communauté zooplanctonique d'eau douce de l'Escaut, décrite par Mialet et al. (2011) pour la période 2007-2009, à plus long terme
2) Une fois le constat confirmé, le second objectif a été d'analyser les facteurs environnementaux ou biologiques qui expliquent le mieux les changements observés sur les communautés zooplanctoniques, et ce sur les trois stations en amont.

II.1.2 Principaux résultats

Dans la partie d'eau douce de l'estuaire entre 2002 et 2012, la qualité de l'eau a continué à s'améliorer. Depuis 2006, la concentration en oxygène présente une amélioration significativement plus élevée. Les concentrations en NH_4-N sont significativement plus faibles après 2006 dans les trois stations. A partir de 2008, nous constatons une augmentation des concentrations moyennes annuelles de Cl^- et SPM. La concentration moyenne annuelle de la Chl *a* est significativement plus faible après 2006 et ce uniquement sur la station 3.

En fonction de ces divers changements environnementaux, nous pouvons classer nos stations selon leur qualité de l'eau. Dendermonde (St 3) est la station ayant la meilleure qualité d'eau, ensuivie d'Uitbergen (St 2) et enfin, de Melle (St 1) avec la qualité d'eau la moins bonne.

En considérant la période 2002-2012, les copépodes calanoides, et plus particulièrement *E. affinis*, sont les plus avantagés par cette amélioration du milieu et ce au détriment des copépodes cyclopoides. L'augmentation de l'oxygène, la diminution de la concentration de NH_4-N ainsi qu'un faible écoulement pendant l'été représentent le « combo idéal » pour expliquer développement d'*E. affinis* dans la partie amont de l'estuaire.

Les changements dans la communauté zooplanctonique ont suivi un gradient induit par l'évolution spatio-temporelle de l'amélioration de la qualité de l'eau. A partir de 2007, la station la plus en aval (St 3) est devenu permissive au développement d'*E. affinis*.

Alors que plusieurs suggestions peuvent être faites pour expliquer la diminution de l'abondance des cyclopoïdes (pâturage concurrentiel, pression de prédation élevée, toxicité du NH3-N, sensibilité à l'oxygène, ...), aucune cause évidente de leur déclin ne peut être avancée.

L'abondance de cladocères dans la zone d'eau douce et saumâtre est restée constante.

II.2 Article 1: Mesozooplankton affinities in a recovering freshwater estuary

Mesozooplankton affinities in a recovering freshwater estuary

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II.2.1 Abstract

Water quality of the Scheldt estuary (Belgium/The Netherlands) has considerably improved in recent years, especially in the upstream, freshwater reaches. Within the zooplankton community, the copepod *Eurytemora affinis*, typically abundant in brackish water and quasi-absent from freshwater before 2007, has since substantially developed in the latter, where it now represents 90 % of the crustacean mesozooplankton community. Simultaneously, cyclopoid copepods have drastically decreased, while cladocerans abundance did not change.

The aim of this study was 1) to verify if the zooplankton community described for the period 2007–2009 by Mialet et al. (2011) has stabilized until present 2) to look for the environmental conditions favouring *E. affinis* development and causing changes in the upstream freshwater zooplankton community. To this purpose, the 2002-2012 temporal evolution of the zooplankton distribution at three stations in the upstream freshwater Scheldt estuary was analysed.

Water quality remained better after 2007 than before, and some factors revealed continuous improvement in annual mean concentrations (e.g. increase in O_2, decrease in BOD5 and NH_4-N concentration). The increase in oxygen and the decrease in NH_4-N concentration, together with low discharge during summer were the main environmental factors explaining the development and timing of *E. affinis* in the upstream freshwater reach. In this reach, *E. affinis* maximal abundance is shifted to higher temperatures (summer) compared to its typical maximum spring abundance peak in the brackish zone of the Scheldt estuary and in most temperate estuaries.

The changes in zooplankton community followed a temporal and spatial gradient induced by the spatio-temporal evolution of water quality improvement. The most downstream station (3) became permissive for *E. affinis* development (oxygen concentration > 4 mg L^{-1}; NH_4-N concentration < 2 mg L^{-1}, discharge (Q) < 50 m^3 s^{-1}) from 2007 onwards, and this station showed the highest *E. affinis* and the lowest cyclopoid abundance. At the more upstream stations *E. affinis* developed later and less strongly, and cyclopoids decreased less in abundance than at station 3.

While several suggestions can be made to explain the decrease in cyclopoid abundance (competitive grazing, high predation pressure, NH_3-N toxicity, sensitivity to oxygen), no clear cause for their decline could be advanced.

Water quality improvement in the freshwater Scheldt estuary has led to environmental, post-heavy polluted conditions, under which no data on zooplankton populations in this estuary were available. This has permitted to detect a plasticity in the temperature tolerance of *E. affinis.*

Keywords:
Scheldt estuary, zooplankton distribution, restoration, *Eurytemora affinis*, oxygen concentration, NH_4-N concentration.

II.2.2 Introcduction

After decades of ecological degradation, many ecosystems benefit from restoration efforts. Also aquatic ecosystems in general and estuarine ecosystems in particular recovered due to better waste water treatment and habitat restoration. The initial objective of restoration to achieve an original ecological status has often been replaced by a more realistic goal. At present, an ecosystem is considered restored when it is able to sustain itself structurally and functionally and consequently to provide ecosystem services (Borja, 2008a,b; Druschke, 2015). Achievement of this goal for severely and long-term polluted systems is a slow and often fluctuating process, which necessitates long-term monitoring to allow adjustment of the restoration and management strategies when necessary (Borja et al., 2010). Indeed, long-term studies allow the detection of latency of biological responses, as it may take time to observe a recovery or a change of communities (Hawkins et al., 2002; Etcheber et al., 2011). However, long-term studies are quite rare because they are expensive and require a lot of material and human effort (Hawkins et al., 2002).

Monitoring of restoration outputs is also a new experience in science, in the sense that, until a decade or two ago degradation of ecosystems was witnessed, not recovery (e.g. Verity 2002a,b; Kemp et al., 2005, Verity & Borkman, 2010; Langsethe et al., 2014). In most cases ecological quantitative monitoring only started after the system was already substantially polluted and consequently little information is available on the pristine or slightly polluted state of system. Hence, it would not be surprising that presently recovering systems face situations that have not been described or quantified yet and reveal some unknown or unexpected ecological relationships. Also, as restoration efforts occur in parallel to ongoing global changes (Anneville et al., 2002, 2009; Verissimo et al., 2013), and estuaries continue to respond to the evolution of multiple user demands, precise effects of environmental factors are often difficult to disentangle.

Many studies on response of estuarine communities focus on large scale aspects, such as hydro-geomorphology, wetland restoration, recovery of top-predator populations (e.g. Orson et al., 1992; Ducrotou & Dauvin, 2008; Beauchard et al., 2011; Maire et al., 2013; Teuchies et al., 2013; Hogg et al., 2014).

Long term monitoring of water quality in estuarine systems are generally focusing on nutrient loads and their management in an eutrophication context. In

general, these studies show that system responses to decreased nutrient loading can differ substantially between systems and between subsystems of an estuary, mainly according to hydrography (e.g. Verity, 2002a,b; Kemp et al., 2005; Boynton et al. 2014; Romero et al., 2016).

However, most papers dealing with estuarine water quality and its effect on pelagic biota report ongoing degeneration of the system rather than restoration, and suggest restoration as a future perspective. For example, Smit et al. (1997) report the evolution in water quality and pelagic community of the Rhine-Meuse Delta after its enclosure in 1970 and give some recommendations (i.e. restoring the estuarine character) for future management. Flaherty et al. (2013) describe the dependence of the nekton community to disturbances of the natural patterns of freshwater delivery to the Florida Bay estuary (USA) by flood-control and water-supply projects and highlight the importance of nekton community monitoring prior to hydrologic manipulations.

Within the pelagic system, phytoplankton and bacteria, as major producers and recyclers, are classically included in biogeochemical studies. Estuarine zooplankton, in spite of being the main trophic link between the primary estuarine resources (i.e. phytoplankton, detritus) and the higher trophic levels (i.e. hyperbenthos, juvenile fishes and some adult fish species) has received little attention. Falcao et al. (2012) report consequences of restoration measures in the Mondego estuary (Portugal) for the zooplankton community. After re-establishment of water circulation between the two branches of this estuary, eutrophication symptoms decreased and higher mesozooplankton density, mainly of estuarine species was observed.

The lack of information on zooplankton response to estuarine restoration is probably due to the minor importance of zooplankton in quantitative energy flow budgets. In addition, contrary to fishes, birds and macrobenthos, the microscopic zooplankton organisms are not readily considered by various stakeholders as a proof of successful management. Also, while phytoplankton can in part be studied by indirect methods (i.e. pigment concentrations, automatic fluorescence monitoring), there are no automated methods used routinely for the evaluation of community composition or activity of zooplankton in estuarine systems. Methods such as Zooscan (Grosjean et al., 2004; Gorsky et al., 2010), applicable in open marine systems or lakes (Schultes et al., 2009; Lelièvre et al., 2012; Marcolin et al., 2013) are

not of use in estuarine systems because of the high suspended matter concentration. Analysing estuarine zooplankton samples thus remains a painstaking task, demanding expertise and patience. Yet, having relatively short lifespan, zooplankton organisms can react rapidly to changing environments (Falcao et al., 2012; Cardoso et al., 2013) and can therefore be considered worthwhile monitoring.

This paper, presents the results of a long-term (11 years) monitoring of the Scheldt estuary, after restoration of water quality from a heavily polluted status since the 1960-1990ties to a less polluted one in the last decades (Heip, 1988; Baeyens et al., 1998; Van Damme et al., 2005; Cox et al., 2009). The Scheldt estuary is a macrotidal estuary covering a marine, brackish and freshwater gradient under tidal influence (Meire et al., 2005; Van Damme et al., 2005) (Fig. 13).

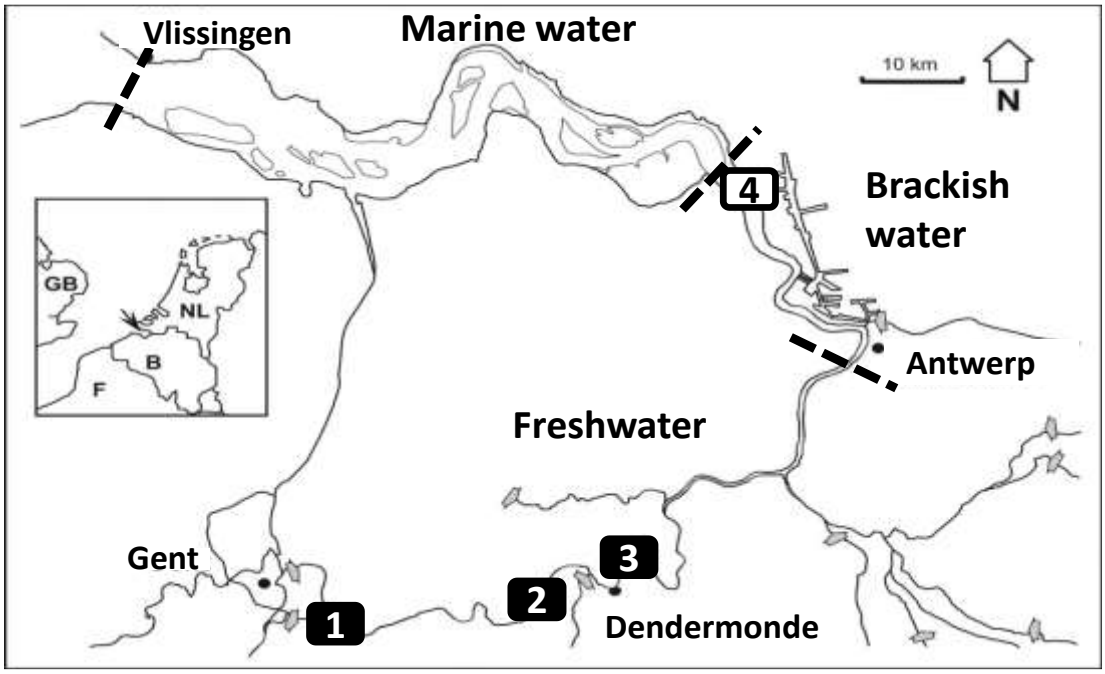


Figure 13 : Map of the Scheldt estuary. Dotted lines indicate limits between marine water, brackish water and freshwater reaches. Freshwater stations considered in this study are represented by black squares: 1- Melle, 2- Uitbergen, 3- Dendermonde. The brackish water station Grens is symbolized by the white square and number 4.

The Scheldt has its source in the North of France and runs through Belgium to join the North Sea at Vlissingen in the Netherlands. Its estuary is situated from the mouth at Vlissingen until the city of Ghent, where the tide is stopped by sluices. The tidal reach between the Dutch – Belgian boarder and the city of Ghent, called the Sea Scheldt, covers 110 km of brackish water and 80 km of one of the few remaining freshwater tidal habitats in Europe (Meire et al., 2005; Van Damme et al., 2005). The

main tributaries entering the Sea Scheldt are the Dender, Durme and Rupel. Contrarily to most temperate estuaries, the Scheldt estuary is characterized by vertically well-mixed water flows (Baeyens et al., 1998), generally showing no salinity or current stratification (Heip, 1988).

The Scheldt estuary has historically been one of the most polluted in Europe (Heip, 1988; Meire et al., 2005). Since two decades, European directives, and specifically the 2000 European Water Framework Directive (WFD), have incited important efforts to increase wastewater treatment capacity on the Scheldt basin and reduce pollutant loads, including organic matter, entering the estuary (Brion et al., 2015). In relation to the upstream reach treated in this paper, the Boven Scheldt watershed wastewater treatment capacity was increased from $2{,}6\ 10^6$ inhabitant equivalents (IE) in 1986 to $5{,}0\ 10^6$ IE in 2014. The capacity on the basin of the Dender, which joins the Scheldt just upstream station 3, increased, during the same period, from $31{,}5\ 10^3$ to $343\ 10^3$ IE (Brion, pers. comm., 2016). As a consequence, oxygen concentrations increased while nutrient concentrations (i.e. NH_4^+-N, PO_4) decreased concomitantly. Since 2009, the morphology of the estuary has also changed due to its deepening between the mouth and Antwerp harbour, leading to increased salinity of the estuary and an increase of the tidal pumping.

In response to the improved water quality, the zooplankton community experienced important changes (Appeltans et al., 2003; Tackx et al., 2004; Mialet et al., 2010, 2011). Between 1996 and 2006, the brackish water community was dominated by calanoid copepods, with the calanoid copepod *E. affinis* being the most abundant species, especially during spring. The freshwater community, (i.e: upstream of Antwerp) was more diverse, dominated by rotifers, cyclopoid copepods and cladocerans. In 2007, a community shift occurred in the freshwater tidal part with *E. affinis* becoming dominant and reaching higher densities than in the brackish part. Concomitantly, cyclopoid copepods decreased to very low abundances. The abundance of cladocerans in the freshwater and brackish water zone remained constant. In the brackish water zone, no significant fluctuations in zooplankton community composition was observed between 1996 and 2009 (Mialet et al., 2010, 2011). Mialet et al. (2011) report changes in the zooplankton community composition in the freshwater observed during three years (2007-2009). Considering natural variability of zooplankton populations in estuarine systems (e.g. Roman et al., 2005;

David et al., 2005; Feike & Heerkloss, 2008) it is necessary to follow up the zooplankton community for a longer period in order to verify the stability of the new situation. Appeltans et al. (2003) and Mialet et al. (2010; 2011) have evidenced the relationship between oxygen concentration (as proxy for water quality) and *E. affinis* development in the freshwater reach of the Scheldt. In recent years, water quality in the freshwater reach is comparable with than in the brackish water reach, but not better. So, in case the 2007–2009 zooplankton community composition should stabilize, the question arises why *E. affinis* can develop so well in the freshwater reach and why cyclopoid copepods decrease in abundance following water quality improvement.

The objectives of the present study are 1) to verify the zooplankton community situation in the Scheldt upstream freshwater reach considering the 2002-2012 period, including five years before and 5 years after the 2007 community shift, and 2) to analyse which environmental or biological factors best explain the observed changes in comparison to the situation prior to 2007.

II.2.3 Material and methods

II.2.3.1 Monitoring

The OMES project, monitors hydraulics, water chemistry and biology (phytoplankton, zooplankton, fishes) and studies march development and interactions between marches and the pelagic compartment of the Sea Scheldt. The OMES results are used in management sustaining ecosystem models (e.g. Cox et al., 2009; Beauchard et al., 2011). OMES monitoring is conducted monthly since 1996 at 17 stations in the main channel and at the mouths of the main tributaries. Since 2012, sampling frequency was intensified to bi-monthly between March and September. A full description of the OMES project is given in Meire (2005) and Van Damme et al. (2005).

Abiotic parameters were collected according to the OMES protocol (Van Damme et al., 2005). At each station and sampling date, surface water samples were collected in the middle of the estuary using bucket hauls from a vessel. For each sampling date several physico-chemical variables were measured: 5-day biochemical oxygen demand (BOD5) using a WTW OXI 96 oxymeter, pH and temperature using a CONSORT C832 electrode and dissolved oxygen concentration (O_2) using a WTWOXI 325, equipped with Clark electrode. Suspended particular matter (SPM) samples were filtered on pre-combusted Whatman GF/C glass fiberfilters. Water samples for quantification of chlorophyll *a* (Chl *a*) concentration was filtered over Whatman GF/F glass fiber filters. Sub-surface water was sampled for the determination of the concentrations of dissolved silica (DSi), chlorine (Cl^-), ammonium (NH_4^+-N), nitrates (NO_3^--N), nitrites (NO_2^--N), orthophosphates (PO_4^--P), and total phosphorous (Ptot) concentrations in the laboratory. Samples were stored at 4°C and analyzed in 24h by colorimetry using a SKALAR SA 5100 segmented flow analyzer. SPM was analyzed by gravimetry, Chl *a* by spectrophotometry. Details of analyzing procedures are given in Van Damme et al. (2005). The Flemisch Administration for Waterways and Maritime Affairs (WNZ) provides daily discharge measures (Q) of the tributaries Boven Scheldt, Dender and Rupel.

For this study, the stations Melle (1), Uitbergen (2) and Dendermonde (3) situated at 155, 140 and 121,5 km from the mouth at Vlissingen respectively (Fig 1), were selected as exclusive freshwater stations, with no or very low influence of salinity (maximum salinity observed at the most downstream station 3: 0.58).

In the Scheldt, seasonal variations in discharge and temperature are more marked in the freshwater zone than in brackish and marine areas. Fresh water discharge shows a seasonal variation, with maximum values in March, declining during April and May to summer minima (Meire et al., 2005, Van Damme et al., 2005). Stations 1 and 2 are situated in the freshwater zone with short residence time (monthly mean residence time 0,3 days in winter, 2,3 days in summer) and station 3 is situated at the beginning of the freshwater zone with long residence time (1,0 in winter, 7,1 days in summer). Stations 1 and 2 receive water from the Boven Scheldt, station 3 receives water from both the Dender and Boven Scheldt. In the following, the zone between station 1 and station 3 will be referred to as 'upstream freshwater zone' (UFZ). For comparing with the brackish water reach concerning *E. affinis* seasonal distribution, the most downstream station of the OMES monitoring, Grens at km 58 (station 4) was used.

II.2.3.2 Sampling of mesozooplancton

Mesozooplankton was sampled at these 4 stations during the monthly (or bi-monthly, between March and September) sampling from 2002 till 2012 by filtering 50 L of sub-surface water through 50 µm mesh plankton net. Zooplankton was fixed in a formaldehyde solution (4 % final concentration). Species were identified and individuals counted under a stereo- (90x magnification) and microscope (960x magnification).

Organisms were identified mainly at the genus level and when possible at the species level. Afterwards, cladocerans and copepods were grouped at suborder and order levels, respectively, to perform statistical analyses, except for the calanoid *E. affinis* that was kept to species level. Counts were converted to number m^{-3}. Abundance data for all months, including those sampled twice, were converted to monthly means.

II.2.3.3 Data analyses

Trends of environmental factors reported are tested for significance using a Spearman rank correlation test at $p<0.05$. Differences between series of observations (for example O_2 concentration at two stations) are considered significant when $p<0.05$ following Mann-Whitney tests. The term "significant" in the text refers to at least this criterion.

The cumulative percentage distributions of mean abundance of *E. affinis* as a function of temperature classes were computed and fitted using Curve Fitting Toolbox of Matlab Software (Mathworks Inc., Version, 7.5).

For all parametric analyses, normal distribution and homoscedasticity were checked. If not respected, variables were log transformed to achieve normal distribution. Multivariate analyses (RDA and GLM) were performed to describe zooplankton communities' distribution in relation to environmental factors using Canoco software (Ter Braak, 1987, 1994).

To explore the heterogeneity in zooplankton community spatio-temporal distribution, a preliminary detrended correspondence analysis (DCA) analysis was run on the taxa dataset. As the inertia was <2.6, a linear distribution is expected so a redundancy analysis (RDA) was performed to explore relationships between environmental factors and zooplankton taxa. Significance of factors was tested by Monte Carlo permutation at $p<0.05$.

Then, generalized linear models (GLM, with Gaussian family) followed by Hierarchical partitioning of variance (HP) using R software (R Development Core Team 2008) (Walsh & MacNally, 2004) were used to identify the main specific environmental predictors of *E. affinis,* cyclopoid copepods and cladocerans abundance in time and space. To optimally take into account the seasonality of occurrence of these organisms, these analyses were done by 3 seasons. Spring was considered from March to May, summer from June to September and winter from October to February. A Hierarchical Partitioning was subsequently used to identify the independent contribution of each explanatory variable by reducing collinearity resulting from correlation between variables. This allows ranking the importance of the covariates in explaining the response variable independently of the other covariates. The proportion of deviance explained by each model (D^2) was calculated according the formula proposed by Guisan and Zimmermann (2000).

II.2.4 Results

II.2.4.1 Temporal evolution of the zooplankton community

Between 2002 and 2007, adult and C5 stages of calanoid copepods were observed in relatively low abundance (with an average 150 ind. m^{-3} with a maximum of 2 000 ind. m^{-3}) in the UFZ. From 2007 to 2012, the mean annual abundance of calanoid copepods strongly increased a maximum of 11 500 ind. m^{-3} at station 3 in 2009 (Fig. 14a). Calanoid copepods abundance was, between 2007 and 2012, higher at station 3 than at station 2 and 1. At the latter station, they became highly abundant (11 000 ind. m^{-3}) in 2011 only and dropped to 840 ind. m^{-3} the following year.

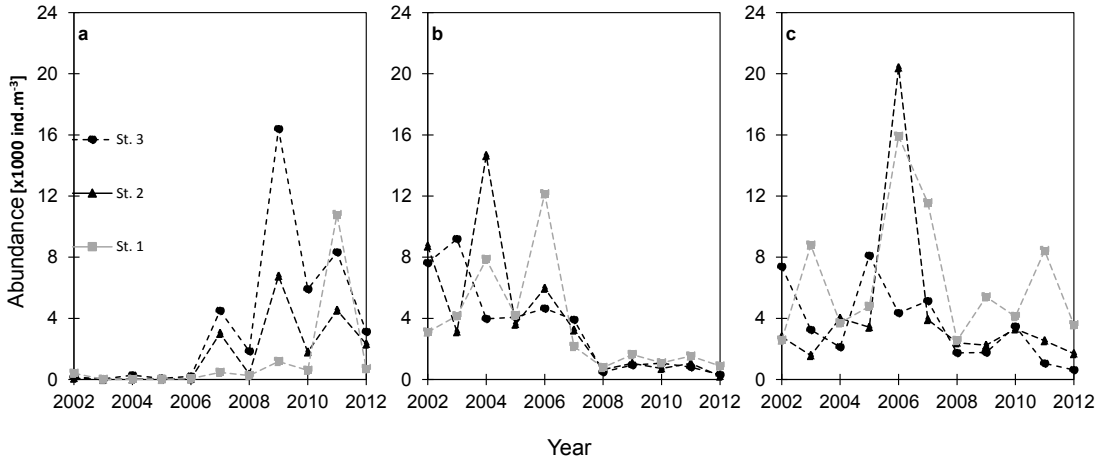


Figure 14 : Evolution of the yearly mean abundance of adult and C5 stages of (a) Calanoid copepods, (b) Cyclopoid copepods and (c) Cladocerans in the Scheldt estuary, at 3 stations. Station 1 (Melle), grey lines), 2 (Uitbergen) and 3 (Dendermonde).

The average annual abundance of adult and C5 stages of cyclopoids (Fig. 14b) varied between 3 000 and 14 700 ind. m^{-3} during the period 2002 to 2007, with a maximum of 14 700 ind. m^{-3} at station 2 in 2004. Cyclopoid abundance declined sharply after 2007, ranging between 220 and 3900 ind. m^{-3}. Cyclopoid abundance fluctuated less after 2007 than before.

Unlike *E. affinis* and cyclopoid abundances, cladoceran mean annual abundance (Fig. 14c) changed little in time during the entire study period with the exception of an mean annual abundance peak (23 400 at station 2 and 15 900 ind. m^{-3} at station 1 in 2006). Both before and after 2007, annual mean cladoceran abundance was higher in station 1 than at the two other stations.

The proportion of *E. affinis*, as a percentage of copepod adults and C5, showed a significant increase during the study period in the UFZ of the Scheldt estuary. Indeed, from 4 % in 2002, it increased up to 50 % in 2007 to further increase up to 70-90 % (spearman rank, $p<0.05$) after 2009 (Fig. 15).

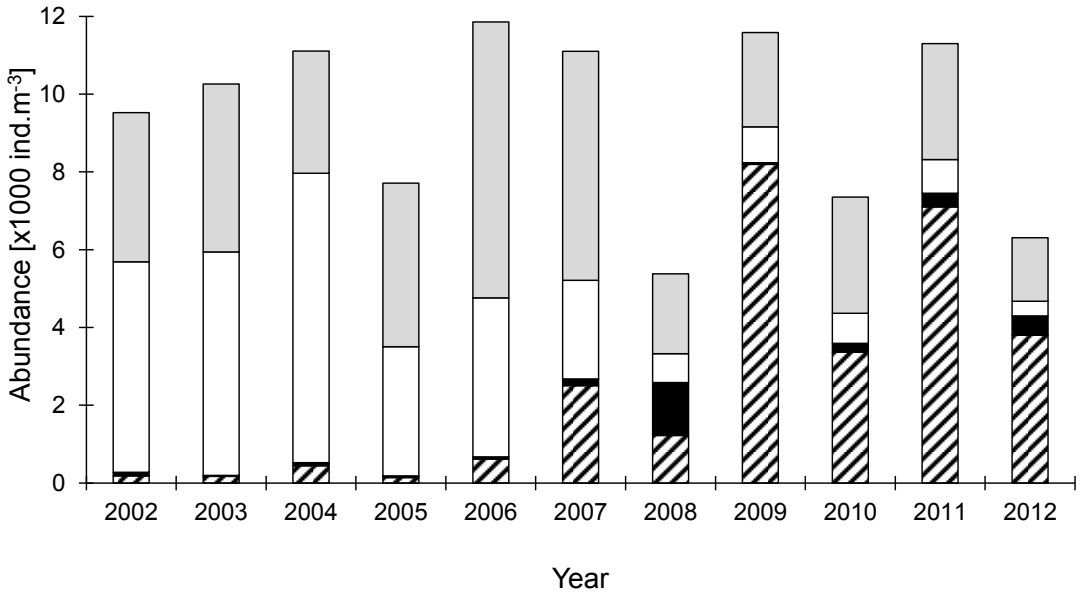


Figure 15: Contribution of different groups to total zooplankton abundance in the upstream freshwater Scheldt from 2002 to 2012.

II.2.4.2 Species abundance – season

An increase in the abundance of *E. affinis* (Fig. 16a) has occurred after 2007, especially when temperatures exceeded 15°C. Thus, in the UFZ, *E. affinis* mainly developed during late spring and summer when temperatures are higher than earlier or later in the year. A high cyclopoid copepod abundance was observed during the years before 2007 with an optimum temperature between 18 and 25°C (Fig. 16b), which corresponds to the summer season.

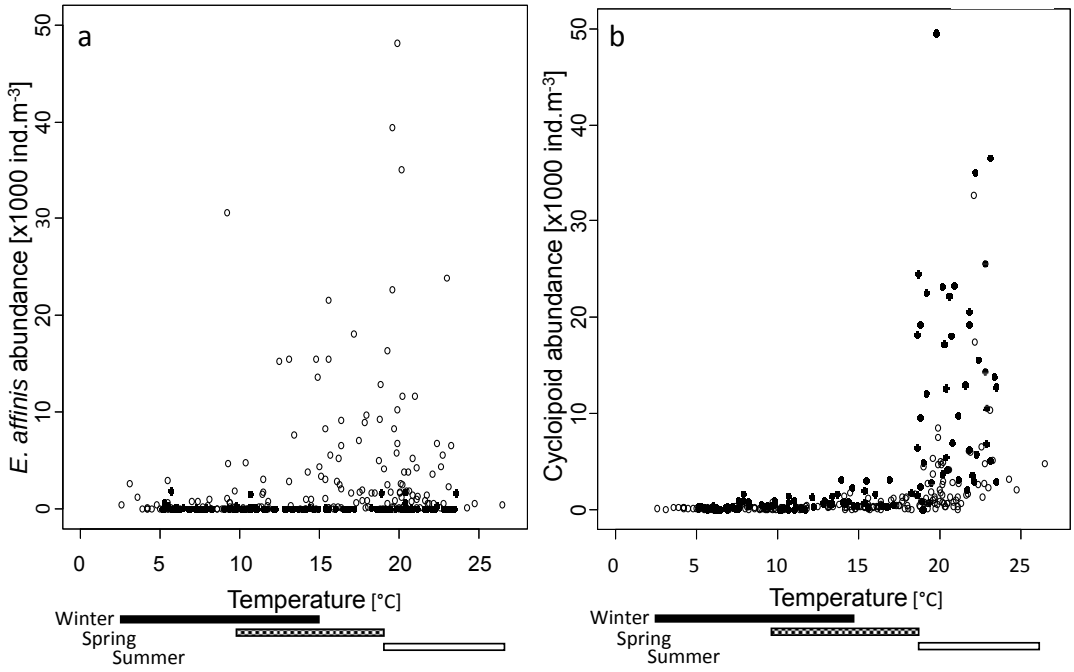


Figure 16 : Relationship between a) *E. affinis* and b) cyclopoid abundance and water temperature at the time of sampling from 2002 to 2012. Bars below the graph indicate temperature range for each season considered.

Distributions of mean abundance of *E. affinis* were symmetrical; with all values of R^2 for Gaussian distributions exceeding 0.98 (Table 1). *E. affinis* shows a clear gradient in temperature of occurrence at the different stations (Fig. 17), with values of μ ranging from 9.56°C at the brackish station 4 until 18.58°C at station 1. μ values for all UFZ stations were significantly higher than the μ value from the brackish water station 4 ($p<0.05$). Standard deviations in the first three stations were not statistically different, which means that the three Gaussian distributions are shifted by μ values that are significantly different ($p<0.05$). However, in the most upstream station 1 both μ and sigma are significantly different from the other distributions ($p<0.05$). The temperature range (i.e. variance) at Melle, the most upstream station, is smaller than the other stations.

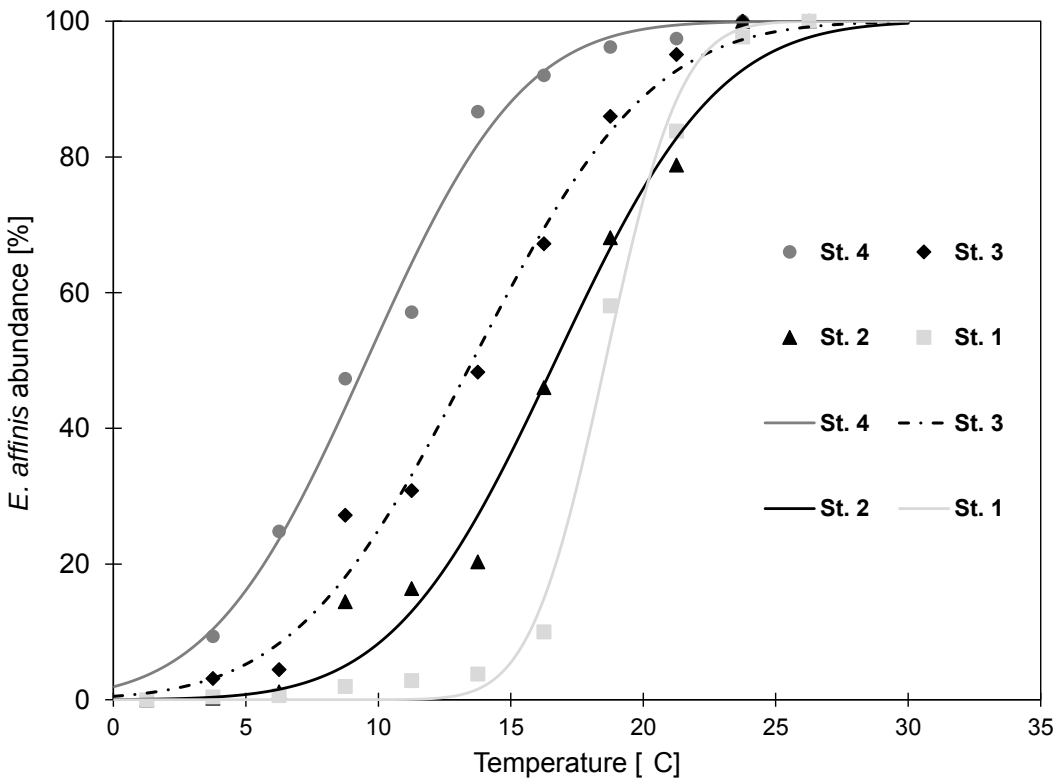


Figure 17 : Cumulative distributions of average abundance of *E. affinis* as a function of temperature classes at the three freshwater stations (1,2,3) and at a brackish water station (4, Grens). The fitted parameters and their associated statistics are shown in Table 1.

Table 1 : Values of the fitted parameters of the normal cumulated density function of average abundance of *E. affinis* and their associated statistics (see also Fig. 5).

	Brackish water	**Freshwater**		
	Grens	Dendermonde	Uitbergen	Melle
R^2	0.9923	0.9916	0.9859	0.9952
μ	9.561	13.56	16.69	18.58
sigma	4.611	5.281	4.827	2.239
μ Coefficients (95% confidence bounds)	9.044-10.08	12.96-14.17	15.96-17.43	18.26-18.89
Sigma Coefficients (95% confidence bounds)	3.874-5.348	4.425-6.136	3.783-5.872	1.79-2.688

II.2.4.3 Water quality evolution

The temporal evolution of the main water quality parameters shows a similar trend during the study period for the three stations (Fig. 18). Between 2002 and 2007, environmental factors characterising water quality in the UFZ showed a downstream-upstream gradient, water quality being worst at station 1 and best at station 3, with station 2 in between. Since 2008, a gradual increase in chlorinity was measured for all 3 upstream freshwater stations, which slightly dropped again in 2012. Some

environmental factors representing water quality remained rather stable after 2007, others continued to improve. Annual mean O_2 concentration was significantly higher since 2006 at all three stations than before, while annual mean BOD5 decreased significantly from 2006 onwards and mean concentrations of NH_4-N were significantly lower at all three stations after 2006 than before. Annual mean concentrations of CL and SPM increased from 2008 onwards. No significant changes in annual mean Chl *a* concentration were observed at stations 1 and 2 over the study period, but at station 3, Chl *a* concentration was significantly lower after 2006 than before. DSi and PO_4-P concentrations stayed rather stable at all three stations (Fig. 18). Water quality was best in the most downstream station 3, worst in the most upstream station 1.

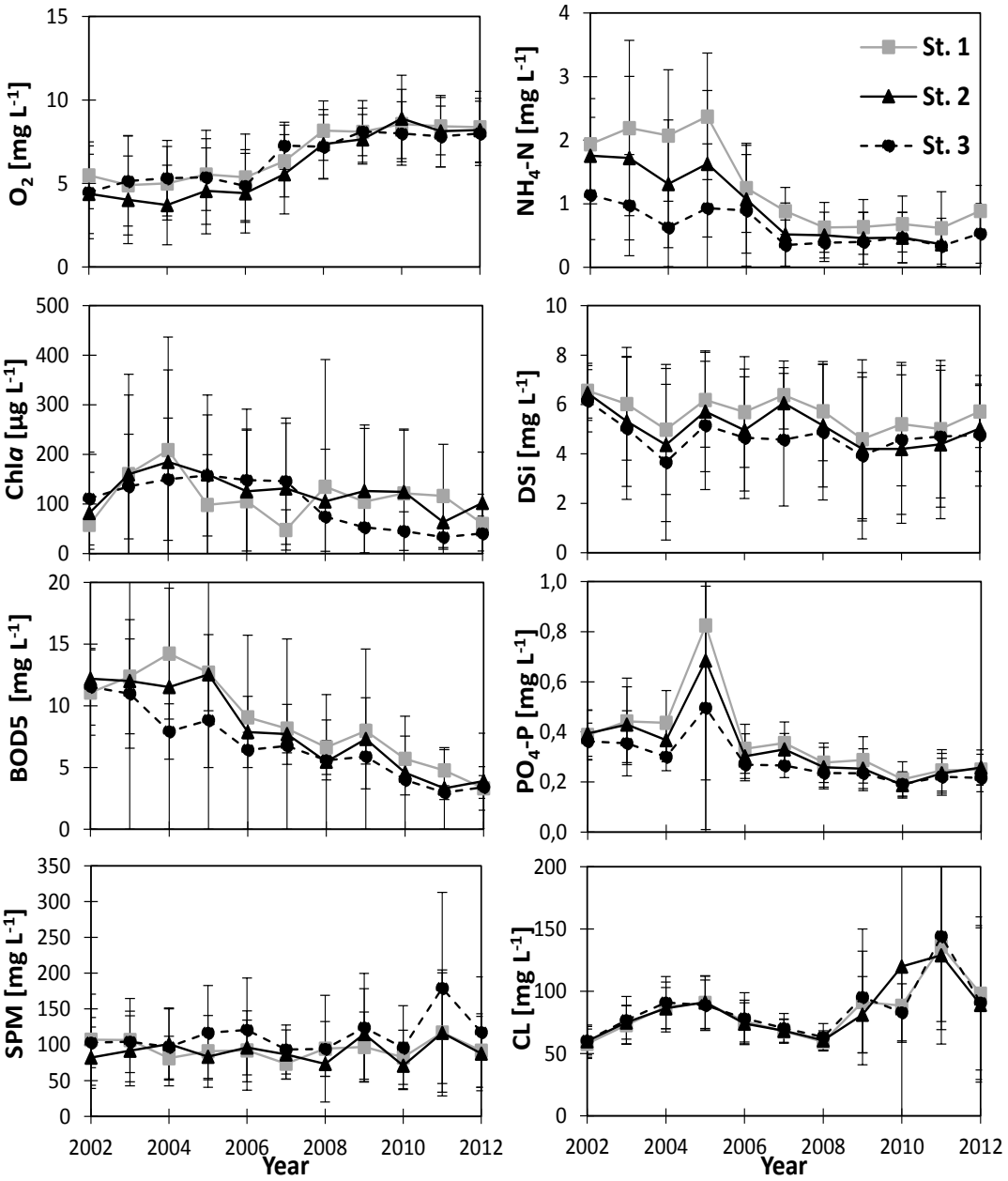
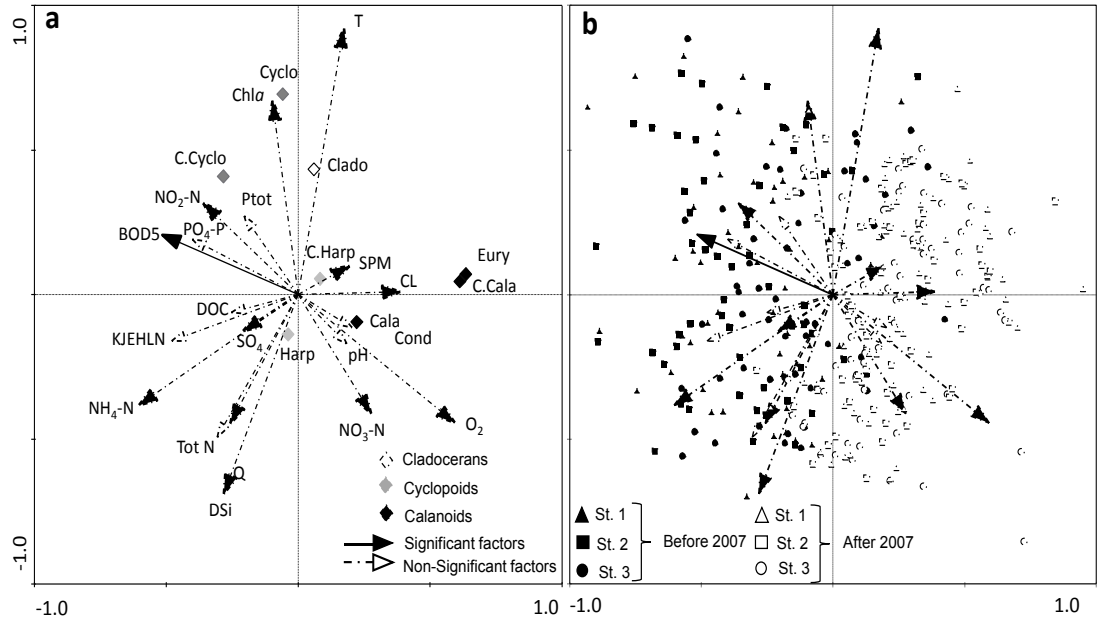


Figure 18 : Evolution of environmental factors in the Scheldt estuary from 2002 to 2012, showing annual mean values. Melle (St1), Uitbergen (St2) and Dendermonde (St3). Vertical bars are standard deviations.

II.2.4.4 Coupling zooplankton species distribution and environmental factors

The first two axes of the RDA analysis accounted for 35 % of the variance of the zooplankton spatio-temporal distribution that was significantly related to (in decreasing significance): temperature, oxygen, BOD5, CL, NH_4-N, discharge, Chl *a,* SPM, NO_3-N, NO_2-N, DSi and SO_4 (Fig. 19; Table 2).



Species	
Eury	Eurytemora affinis
Cala	Calanoids except *E. affinis* (adult + C\
C.Cala	Copepodits Calanoids
Cyclo	Cyclopoids (adulte + CV)
C.Cyclo	Copepodits Cyclopoids
Harp	Harpacticoids (adulte + CV)
C.Harp	Copepodits Harpacticoids
Clado	Cladocearans

Axes	1	2	3	4
Eigenvalues	0.205	0.095	0.012	0.006

Sum of all canonical eigenvalues : 0.325

Figure 19 : Redundancy analysis (RDA): taxa-environmental factors 1,2 (a, b) biplots in the upstream freshwater Scheldt for the 2002-2012 period.

Table 2 : Results of the redundancy analysis (RDA) testing the effects of abiotic factors on the abundance distribution of mesozooplankton in the upstream freshwater Scheldt. Factors are listed by their eigenvalues (λ), i.e., the contribution of each factor to the explanation of zooplankton abundance, without covariability (see Methods). *, **, * indicate factors that were statistically significant (Monte Carlo permutation test at p< 0.05, p< 0.01, p < 0.001 respectively).**

RDA conditional effects			
Factors	λ	F-value	p-value
T	**0.1**	**43.72**	**0.001*** **
O_2	**0.09**	**35.42**	**0.001 *** **
BOD5	**0.03**	**11.16**	**0.001*** **
CL	**0.02**	**8.97**	**0.001*** **
NH_4-N	**0.01**	**6.4**	**0.001*** **
Q	**0.02**	**5.86**	**0.001*** **
Chl*a*	**0.01**	**5.41**	**0.003** **
SPM	**0**	**3.41**	**0.010** **
NO_3-N	**0.01**	**2.53**	**0.040***
NO_2-N	**0**	**3.02**	**0.014***
Dsi	**0.01**	**2.6**	**0.031***
SO_4	**0**	**2.43**	**0.042***
PO_4-P	0.01	2.01	0.079
DOC	0.01	1.53	0.153
pH	0	1.63	0.155
Ptot	0	1.31	0.235
Ntot	0	1.29	0.262
KJEHLN	0	1.15	0.312
Conductivity (Cond)	0	1.13	0.361
Sum of all λ	0.32		

The geometric representation of zooplankton abundances and environmental factors in the plan defined by the first two axes indicates that the distribution of *E. affinis*, calanoid copepodites, and to a lesser extent calanoid adults/C5 are associated with oxygen concentration, NO_3-N, CL and SPM, and perpendicular to the temperature and Chl *a* vectors (Fig. 19a). Most other environmental variables are in opposite direction towards the left hand side of the biplot. Cyclopoid adults/C5 and copepodites, as well as cladocerans are associated with increasing temperature and Chl *a* concentration (Fig. 19a). A clear separation on the second and third axes of the RDA between samples before and after 2007 was highlighted (Fig.19b), the latter

being situated at the opposite of poor water quality indicators (NH_4-N, NO_2-N, and BOD5).

In order to eliminate the seasonal influence, GLM analysis aiming at detecting the specific factors influencing *E. affinis*, cyclopoids and cladocerans specifically, were run for each season separately. In view of the change in timing of *E. affinis* maximal abundance between the UFZ and the brackish water zone and between stations within the UFZ, the complete spring-summer period was also considered. While showing the complete results in Table 3. For clarity, only the seasons of maximum abundance of each zooplankton taxa, and factors being significant predictors at a significance level of at least $p< 0.01$(ANOVA) are discussed here.

Table 3 : Results derived from the hierarchical partitioning and generalized linear models performed on *E. affinis*, cyclopoids copepods and cladoceran abundance responses, during the three seasons considered and during the combined spring-summer season. Variables shown for each multiple regression were kept after a stepwise procedure of variable selection. The relative independent contribution of each environmental predictor is given as a percentage of the total independent contribution. Significance following ANOVA testing is indicated as *, **, * for p< 0.05, 0.01 and 0.001 respectively. Minus signs between brackets show negative regression coefficients in the GLM analysis. The D-squared of generalized linear models is the equivalent of the R-squared value of linear models that measures the proportion of variation that the model accounts for. AIC is a model comparison test, to select the "best"model. Ni: not included in the model, ns: not significant.**

Eurytemora affinis			Cyclopoïds				Cladocerans			
Spring	Summer	Spring-Summer	Winter	Spring	Summer	Spring-Summer	Winter	Spring	Summer	Spring-summer
53.99	53.66	47.04	45.32	50.77	50.42	47.75	49.99	59.05	31.41	24.23
ni	ni	ni	16.09***	25.01***	22.75***	6.91***	ni	ni	78.55***	ns
20.57*** (-)	ns	5.43 ** (-)	13.77*** (-)	21.35*** (-)	4.89*	16.67***(-)	32.84***(-)	12.68***(-)	19.13*	ni
5.89*** (-)	10.48** (-)	5.45* (-)	39.52***	44.26***	ni	20.76***	ns	30.50***	ni	31.96***
ni	7.29***	9.3*	4.6* (-)	ni	ni	ni	ni	ni	ni	ni
ni	ni	ni	ni	ns	ni	3.06***(-)	21.82***(-)	ns	ni	ni
49.81*** (-)	6.47*** (-)	18.84*** (-)	ni	ns	ni	ni	ni	ns	ni	ni
ni	8.01*	ni	19.53**	ni	ni	ni	15.53***	18.92**	ns	32.23***
ni	ni	ni	ni	ni	ni	ni	ns	ns	ni	8.65**
23.71**	36.58***	27.84***	ni	ni	23.74*** (-)	19.56***(-)	3.44**	ni	ns	ni
ni	25.28***(-)	22.94* (-)	6.45* (-)	ni	16.72*	11.71*	ni	ni	ni	ni
ni	ni	6.16*** (-)	ni	9.37** (-)	ni	5.24***(-)	26.37***(-)	10.47**(-)	ni	ni
ni	ns	4**	ni	ni	26.74*** (-)	ns	ns	27.50***(-)	2.31**(-)	23.37**(-)
ni	5.86**	ni	ns	ns	5.54* (-)	ni	ni	ns	ni	ni

During spring and summer, the two most important seasons for *E. affinis*, the selected GLM model explained 53.99 and 53.66 % of the total deviance of the *E. affinis* abundance respectively (Table 3). The best predictors were the NH_4-N (negative) and O_2 concentrations (positive), which independently contributed to 49.81 % (spring) and 6.47 % (summer) for NH_4-N and 23.71 % (spring) and 36.58 % (summer) for O_2. Discharge was a significant negative predictor of the *E. affinis* abundance in spring and when considering the spring and summer period together, but contributed less than O_2 or NH_4-N. Cyclopoid copepods were mainly abundant in summer. In this season, DSi and O_2 concentrations were the most significant negative predictors of cyclopoid abundance, independently contributing to 26.74 %, 23.74 %,

while BOD5, PO_4-P and discharge were significant positive predictors of cyclopoid abundance, explaining 22.75 %, 16,72 and 4,89 % of the deviance, respectively. The selected model explained 50.42 % of the total deviance of the cyclopoid abundance (Table 3). When considering spring and summer together, O_2 concentration and discharge were significant negative predictors for cyclopoid abundance (19,67 and 16,67 % respectively), while and Chl *a* concentration (20,76 %), PO_4-P concentration (11,71 %) and BOD (6,91 % were the strongest positive predictors of cyclopoid abundance.

Like cyclopoids, cladoceran abundance was most important in summer. GLM showed the importance of two major factors explaining cladoceran abundance in summer, namely BOD5 (78.5 %) and discharge (19.13 %). The selected model explained 52.62 % of the total deviance of cladoceran abundance. Considering the spring–summer period, Chl *a* concentration (31,96 %) and NO2-N concentration (32,23 %) predicted cladocerans abundance positively, while Dsi concentration predicted it negatively (23,37 %). Discharge was no significant predictor of cladocerans abundance during the spring–summer period.

E. affinis abundance at all three UFZ stations, plotted as a function of the three main predictors (O_2 and NH_4-N concentration, and discharge) resumes the development conditions for this copepod *E. affinis* could develop high abundances (>3000 ind m^{-3}) in a space limited between O_2 >4mg L^{-1} and NH_4-N concentrations < 2 mg L^{-1}. However, between 1 and 2 mg NH_4-N L^{-1}, abundance values > 3 000 ind. m^{-3} were rare. This was also the case at discharge values > 50 m^3 s^{-1} (Fig. 20).

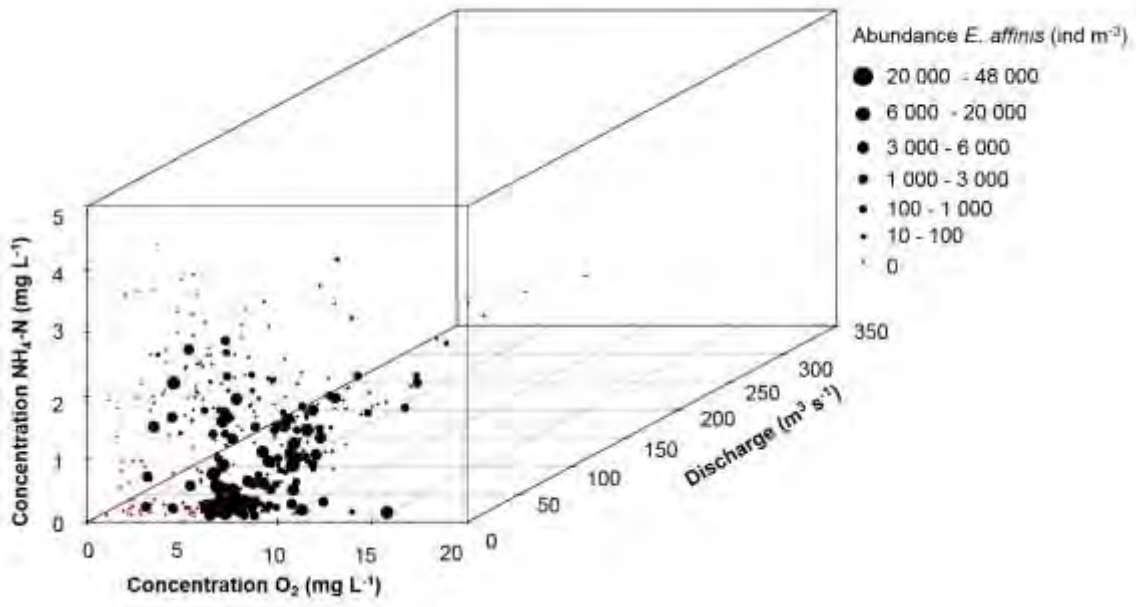


Figure 20 : Distribution of *E. affinis* abundance in the upstream freshwater Scheldt estuary between 2002 and 2012 as a function of O_2, NH_4-N concentrations and discharge.

II.2.5 Discussion

II.2.5.1 Temporal evolution of water quality

The gradual and obvious trend towards a better water quality of the Scheldt already observed in the nineties (Van Damme et al., 2005, Mialet et al., 2010) has continued in recent years. The major changes observed in this study are a decrease in concentrations of NH_4-N, BOD5, PO_4, NO_2 and an increase in O_2 concentrations. While before 2007 yearly mean concentrations of water variables typical of poor quality such as NH_4-N, BOD5, PO_4, and NO_2 were significantly higher at the most upstream site (station 1) than downstream (station 3), values became more similar between the 3 stations after 2007. We here consider these main changes in environmental conditions only as a background to interpret the evolution of the zooplankton community. Details and in depth discussion about two decades of water quality evolution in the Sea Scheldt, and comparison with other European estuaries, will be presented in another paper (Maris et al., in press).

II.2.5.2 Possible causes of E. affinis' abundance increase in the UFZ

The 2010–2012 monitoring period showed that *E. affinis* has continued to increase its presence in the UFZ, becoming highly dominant within the copepod community since 2009. *E. affinis* has previously been reported as a 'freshwater-invader' in various types of freshwater habitats in North America, Asia and Europe within the past century (Bowman & Lewis, 1989; Lee et al., 2003, for a summary), probably due to its ability to cope with low salinity by adapting its osmoregulatory system rapidly (Lee, 1999; Lee et al., 2012). As such, the colonization of the freshwater area of the Scheldt by *E. affinis* is not surprising. Yet, it raises a number of interesting questions with regard to the consequences of restoration measures upon the zooplankton community abundance and composition, especially when considering the strong decrease in cyclopoid abundance that has occurred concomitantly. During the seasons of highest abundance of *E. affinis* (spring and summer), oxygen and NH_4-N concentrations contributed most in explaining the variation in its abundance. Appeltans et al. (2003) and Mialet et al. (2010; 2011) highlighted that *E. affinis*' abundance in the upstream Scheldt (i.e. salinity < 0.5) is conditioned by oxygen concentration. Between 1996 and 2009, *E. affinis* was only abundant at salinity > 0.5 when O_2 concentration in the upstream area was > 4 mg L^{-1} and > 1.3 mg L^{-1} in the middle part of the Scheldt (km 82–110).

The GLM analysis has shown that, discharge also (negatively) contributes to explaining *E. affinis* development in the UFZ, but less strongly than oxygen and NH_4-N concentration. Nevertheless, low summer discharge and hence longer residence times (2,3- 7,1 days) during summer may have favored *E. affinis* the development in the UFZ in addition to improved water quality. In a situation of particularly low spring flow, Kimmel et al. (2006) observed a rise in the abundance of *E. affinis* in freshwater areas of the Chesapeake Bay during the spring.

From 2007, oxygen concentration in all 3 UFZ stations has been, on most occasions, higher than 4 mg L^{-1}, the threshold value advanced by Mialet et al. (2011) as permissive for *E. affinis* development. With regard to oxygen concentration, the UFZ was suitable for the development of *E. affinis* since 2007. In concert with increasing oxygen concentrations, ammonia concentrations have decreased in the UFZ over the study period. Months with NH_4-N concentrations > 1 to 2 mg L^{-1} are mainly winter months, as mineralisation is slower at low temperature. In the brackish water area, where *E. affinis* has always been abundant, ammonia concentration has

always been lower than 1 mg L^{-1} (OMES, unpublished data). In the UFZ, 2007 was the first year with no (station 3) or few (stations 2, 1) months with O_2 < 4 mg L^{-1} and/or NH_4-N > 1- 2 mg L^{-1} between June and October (not shown). This could explain the quite sudden development of *E. affinis* in the UFZ from this year onwards. The fact that O_2 and NH_4-N conditions enabling the development of *E. affinis* occurred later during long periods at stations 2 and 1 than at station 3 may also explain why *E. affinis* developed later and less importantly at these two former stations, especially at station 1, than at station 3. As to seasonality, months with NH_4-N concentrations > 1 mg L^{-1} are winter months, as mineralisation is slower at low temperature.

This suggests that temperature could be the limiting factor for *E. affinis* development in the UFZ. However, comparison with *E. affinis* abundance > 2 900 ind. m^{-3} in brackish waters (i.e. station 4) shows that *E. affinis* can be present earlier in the year since there, its peak abundance was reached during March (at a temperature of 9.8°C), while in the UFZ, *E. affinis* abundance peaked between March and June. In most temperate estuaries, *E. affinis* is known as a spring species (Heinle & Flemer, 1975; Sautour & Castel, 1995; Gasparini et al., 1999; Devreker et al., 2009). Apparently, its development in the UFZ implied a change in temperature distribution from a temperature corresponding to the yearly median population abundance of 9.56°C in brackish water to 13.56 -18.58°C in UFZ. In the UFZ, the decrease in discharge from spring to summer has probably also contributed to *E. affinis* maximum development during summer rather than spring. Within the UFZ, a tendency for later and less strong *E. affinis* development was manifested in upstream direction, as shown in Fig. 5. This could also reflect the shorter residence times in upstream direction, hampering *E. affinis* development upstream. Although chlorinity has increased in the UFZ since 2009, potentially favouring *E. affinis* development, chlorinity in the former remained much lower than in brackish water zone (OMES, unpublished data).

Although no increase in the mean annual Chl *a* concentration was observed over the study period, primary production has increased in the UFZ following water quality improvement (Cox et al., 2009). Chl *a* concentration is generally higher in the UFZ of the Scheldt than in the brackish water zone (Van Damme et al., 2005). Hence, a higher amount of phytoplankon available might also have stimulated *E. affinis* to

extend upstream. *E. affinis* has indeed been reported to benefit from food concentration to increase its salinity tolerance (Lee et al., 2013; Hammock et al., 2015).

II.2.5.3 Possible cause of the evolution in cycloipoid and cladoceran abundance

Contrary to *E. affinis*, cyclopoid mean annual abundances were lower after than before 2007. Thus, it seems that cyclopoids did not benefit from the improved water quality in the UFZ.

During the season of their highest abundance (i.e. summer), cyclopoids were mostly associated with BOD5 and negatively related to DSi and O_2 concentrations. The negative correlation with DSi is a seasonal phenomenon as the summer peak of cyclopoids occurs each year between the fall of Dsi concentration caused by diatom blooms and the rise of the DSi concentration in autumn (not shown). The fact that UFZ residence times are increased during summer (up till a mean of 2,31 days at station 1 and 2 and 7,08 days at station 3) probably profited cyclopoid development during this season both before and after the 2007 shift.

A possible explanation for the decrease in cyclopoid abundance is a direct or indirect negative impact of *E. affinis* on other zooplankton species. While the invasion by *E. affinis* into freshwater systems has been well documented, few studies have considered the parallel evolution of the whole mesozooplankton community. From those rare studies, results suggest that, in most systems where *E. affinis* or other *Eurytemora* spp. co-occur with other zooplankton species, *E. affinis* is highly dominant (Counahan et al., 2005; Vad et al., 2012). Predation by *E. affinis* on (young) cyclopoids and cladocerans could reduce the size of these populations. Nevertheless, this seems rather unlikely, as fatty acid analysis carried out on *E. affinis* females collected from the 0.5-1.8 salinity range in the Scheldt during 2007-2008 suggested a dominant contribution of phytoplankton versus terrestrial or heterotrophic inputs in *E. affinis'* diet (Mialet et al., unpublished). Another fatty acid analysis performed on *E. affinis* sampled in 2005 for the same season and area (Van den Meersche et al., 2009) reflected a slightly more carnivorous but still phytoplankton dominated regime for *E. affinis*. Grazing experiments conducted in 2013-2014 also showed an important grazing activity by *E. affinis* (Chambord et al., unpublished).

Contrary to the observed increasing trend of *E. affinis* abundance and decreasing trend of cyclopoids abundance over the study period, the cladoceran abundance did not show any significant temporal trend in any of the 3 stations studied. Cladocerans, like cyclopoids, were found to be mostly associated with BOD5 and Chl *a* concentration and seem to thrive well in poor water quality conditions. But, in contrast to cyclopoid copepods, cladocerans seem to have been unaffected by the improvement in water quality in the UFZ. Cladocerans have the ability to produce extracellular haemoglobin (Hb) at low oxygen concentrations (Sell, 1998; Pirow et al., 2001 and earlier references therein), which explains they can thrive in eutrophic and oxygen poor environments such as the freshwater Scheldt before 2007. In copepods, the presence of this haemoglobin is scarcely documented. As far as we know, it has only been reported in a few species collected from mud dwelling and borrow habiting harpacticoids (Fox, cited by Green, 1957) and in parasitic siphonostomatoids of deep hydrothermal vent environments (Sell, 2000). Given the importance of eutrophication issues worldwide, studies about oxygen metabolism and oxygen tolerance have mainly dealt with hypoxia. Physiological studies on differences in low oxygen tolerance and metabolism between cladocerans and copepods and between calanoid and cyclopoid copepods are thus clearly needed.

An indirect consequence of high oxygen concentration is the formation of unionized ammonia (NH_3-N), which is more toxic than NH_4-N for organisms. Arauzo and Valladolid (2003) have found that, in waste water treatment ponds, a population of 2 zooplankton species (the cladoceran *Moina micrura* and the rotifer *Brachionus rubens*) showed high mortality rates during periods of increased pH and temperature leading to the formation of NH_3-N. In the Scheldt UFZ, following the increase of primary production pH has risen between 2002 and 2008 (Cox et al., 2009), but combined with the NH_4-N concentration decrease, calculated NH_3-N concentrations were around 0,01 mg L^{-1} and decreasing to 0,001 mg L^{-1} between 2002 and 2012. These concentrations are way below the NH_3-N LC_{50} values for crustaceans which vary from 0,3– 4,4 mg L^{-1} (Ostrensky et al., 1992).

While fish populations have been re-colonizing in the freshwater Scheldt (Breyne et al., 2011), there is no obvious reason why cyclopoids would be more heavily predated than *E. affinis*. This copepod has been shown to be a major food source for (larvae and/or adults of) sprat (*Sprattus sprattus*), herring, goby, smelt

(*Osmerus eperlanus*), flounder and striped bass in several estuarine systems (Nobriga, 2002, Martino and Houde, 2010, Mehner, 2011), among which the brackish water Scheldt (Maes et al., 2005). Nevertheless, Lankov et al. (2010) found that in the Gulf of Riga, where the zooplankton community included the cladoceran *Bosmina longispina*, rotifers *Keratella cochlearis* and *K. quadrata* and the copepod *E. affinis*, several fish species such as adult sprat, juvenile smelt and the three spined stickeback *Gasteostrus aculeatus* were mainly feeding on *B. longispina* rather than on other species. But they also highlighted that the zooplankton abundance dynamics did not reflect fish predation selectivity, suggesting that fishes may switch prey as a function of the zooplankton community composition. Byron et al. (1984), report that, in the oligotrophic Lake Tahoe (USA), the cladoceran *Bosmina longisrostris* is more heavily predated by the omnivorous calanoid copepod *Epischura nevadensis* than the calanoid copepod *Diaptomus tyrrellii*. Viitasalo and Rautio (1998), observed escape reactions of cladocerans and *E. affinis* to mysid predation and suggested that copepods have higher escape abilities and need to be actively attacked while cladocerans can be collected by filter-feeding.

Competition for resources could also explain the observed changes in zooplankton distribution. Mialet et al. (2011) studied the entire OMES sampling transect from Ghent to the Dutch/Belgium border and found that cyclopoids are positively related to Chl *a* concentration. This could however be due to a spatial effect, as Chl *a* concentration in the freshwater zone, where cyclopoids were abundant before 2007, was always higher than in the brackish water zone. Yet, in the present study, considering only the upstream freshwater zone, cyclopoids are also associated with the Chl *a* concentration. Before 2007, cyclopoids in the UFZ reached highest abundance (> 10 000 ind. m^{-3}) during June-August at Chl *a* concentrations between 100-280 µg L^{-1}. After 2007 during these months, the Chl *a* concentration decreased to median concentrations of 89 µg L^{-1} at station 3, 202 µg L^{-1} at station 2 and 192 µg L^{-1} at station 1. It is possible that phytoplankton biomass has become too low to enable cyclopoids feeding efficiently, at least at station 3 and this decrease could be caused by *E. affinis* grazing, but phytoplankton biomass decrease cannot explain cyclopoid abundance decrease at the more upstream stations. The decline in phytoplankton biomass at station 3 may also be induced by an increased turbidity due to increased tidal pumping in the last years (OMES, unpublished data). In addition, other food

resources than phytoplankton need to be considered. BOD5, indicative of detritus and the associated microbial community (e.g. bacteria) was one of the significant predictors for cyclopoid abundance. Some rotifer species, such as *K. cochlearis*, *Filinia longiseta*, *Lepadella* sp., which can be abundant in the Scheldt, can efficiently consume bacteria (Bogdan & Gilbert, 1984; Sanders et al., 1989; Ooms-Wilms et al., 1995). Cladocerans feed on bacteria with more or less limited effect (Borsheim & Andersen, 1987; Güde, 1989). Cyclopoids are known for their omnivory and can thus affect the bacterial abundance (Dobberfuhl et al., 1997). In the UFZ the decrease of BOD5 may have resulted from a decrease of microbial abundance, representing a resource limitation for cyclopoids. *E. affinis,* being mainly herbivore, would rather benefit from increased primary production.

The results of this study support the importance of long-term monitoring programs. Indeed, the long-term monitoring of the restoring Scheldt estuary has highlighted the response of the mesozooplankton taxa to water-quality improvement. The overall trend is that the reduction of organic load enabled the upstream Scheldt estuary to change from a hypereutrophic system in which phytoplankton primary production was inhibited to a eutrophic system with substantial increase in primary production (Cox et al., 2009). Subsequently, these changes led to suitable conditions for *E. affinis* in this stretch of the estuary. It is worth noting that not only the re-oxygenation of the system (i.e. O2 concentrations > 4 mg L^{-1}) improved the colonization of *E. affinis* in the upstream freshwater Scheldt, but also a decrease of NH_4-N concentration below a threshold of 1 mg L^{-1}. As far as we are aware, the studies on the Modego (Falcao et al., 2012) and the Scheldt (Mialet et al., 2011) zooplankton communities are pioneer studies reporting the evolution of an estuarine zooplankton community in parallel to water quality restoration. In the Scheldt estuary, the change of the freshwater reach from hypereutrophic to eutrophic has created conditions under which no previous data on zooplankton have been published. The oldest zooplankton data for the freshwater Scheldt cover the 1967-'69 period (De Pauw 1975). During this period, oxygen concentration during spring in the freshwater reach was below the 4 mg L^{-1} limit for *E. affinis* to develop in this area. At that time, *E. affinis* was mainly found in the brackish part of the Scheldt estuary, as in the 1996-2007 period. Hence, the oxygenation of the upstream Scheldt being an environmental situation closer to pristine conditions than ever inventoried in Scheldt zooplankton

studies, it seems possible that *E. affinis* is not an invader but in fact a returning fugitive. It would thus be interesting to investigate how far improved water quality was also involved in other freshwater systems invaded by *E. affinis*. The change in environmental conditions has also permitted to discover a capacity of *E. affinis* to adapt to temperature ranges for maximum development. This copepod, so far considered as a typical spring species, can reach higher abundances during summer in the Scheldt UFZ than ever observed during spring in the brackish water zone.

Explaining the observed negative effect of the improved water quality on cyclopoids since 2007 is less obvious.

It is important to realize that total mesozooplankton abundance is not higher after 2007 than before. Only the composition of the mesozooplankton community has changed. Does this composition represent a better functioning ecosystem? It will have to be verified in how far the present, apparently stabilizing mesozooplankton community assures its trophic function (i.e. to shuttle energy from the primary producers and the detritus to be higher trophic levels) before the dominant taxa occurring in this community (*E. affinis*) can be evaluated as a water quality indicator.

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II.2.7 References

A --

Anneville, O., Ginot, V., Angeli, N, 2002. Restoration of lake Geneva: Expected versus observed responses of phytoplankton to decreases in phosphorus. Lakes & Reservoirs: Research and management 7, 67-80.

Anneville, O., Souissi, S., Molinero, J.C., Gerdeaux, D, 2009. Influences of human activity and climate on the stock-recruitment dynamics of whitefish, Coregonus lavaretus, in Lake Geneva. Fisheries Management and Ecology 16, 492-500.

Appeltans, W., Hannouti, A., Van Damme, S., Soetaert, K., Vanthomme, R. & Tackx, M., 2003. Zooplankton in the Schelde estuary (Belgium/The Netherlands). The distribution of *Eurytemora affinis*: effect of oxygen? Journal of Plankton Research 25, 1441-1445.

Arauzo, M., Valladolid, M., 2003. Short-term harmful effects of unionised ammonia on natural populations of *Moina micrura* and *Brachionus rubens* in a deep waste treatment pond. Water Research 37, 2547-2554

B --

Baeyens, W., Van Eck, B., Lambert, C., Wollast, R., Goeyens, L., 1998. General description of the Scheldt estuary. Hydrobiologia 366, 1-14.

Beauchard, O., Jacobs, S., Cox, T.J.S., Maris, T., Vrebos D., Braeckel, A.V., Meire, P., 2011. A new technique for tidal habitat restoration: Evaluation of its hydrological potentials. Ecological Engineering 37(11), 1849-1858.

Bogdan, K. G., Gilbert, J. J., 1984. Body size and food size in freshwater zooplankton, Proc. Natl. Acad. Sci. U. S. A. 81, 6427-6431.

Borja, A., Dauer, D.M., 2008. Assessing the environmental quality status in estuarine and coastal systems: Comparing methodologies and indices. Ecological Indicators 8(4), 331-337.

Borja, A., Bricker, S.B., Dauer, D.M., Demetriades, N.T., Ferreira, J.G., Forbes, A.T., Hutchings, P., Jia, X., Kenchington, R., Marques, J.C., Zhu, C., 2008. Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide. Marine Pollution Bulletin 56(9), 1519-1537.

Borja, Á., Dauer, D. M., Elliott, M., Simenstad, C. A., 2010. Medium-and long-term recovery of estuarine and coastal ecosystems: patterns, rates and restoration effectiveness. Estuaries and Coasts, 33(6), 1249-1260.

Børsheim, K.Y., Andersen, S., 1987. Grazing and food size selection by crustacean zooplankton compared to production of bacteria and phytoplankton in a shallow Norwegian mountain lake. Journal of Plankton Research 9, 367-369.

Bowmann, T.E., Lewis, J.J., 1989. Occurrence of the calanoid copepod *Eurytemora affinis* (Popper) in the Ohio river at Louisville, Kentucky. Journal of Crustacean Biology 9(1): 83-84.

Boynton, W.R., Hodgkins, C.L.S, O'Leary, C.A., Bailey, E.M., Bayard, A., Wainger, L.A, 2014. Multi-decade responses of tidal creek system to nutrient load reductions: Mattawoman creek, Maryland USA. Estuaries and Coasts 37(1), S111-S127.

Breyne, J., Maes, J. Ollevier, F., Stevens M., 2011. Fish assemblages across a salinity gradient in the Zeeschelde estuary (Belgium). Belgian Journal of Zoology 141, 21-44.

Brion, N., Verbanck, M. A., Bauwens, W., Elskens, M., Chen, M., Servais, P., 2015. Assessing the impacts of wastewater treatment implementation on the water quality of a small urban river over the past 40 years. Environmental Science and Pollution Research, 22(16), 12720-12736.

Brion, 2016. Data from wastewater management agencies in Wallonia (Société publique de gestion de l'eau - SPGE), Brussels (Société Bruxelloise de gestion de l'eau - SBGE), Flanders (Aquafin) and France (Ministére de l'environnement, de l'énergie et de la mer – Artois Picardie) assembled by Brion N.

Byron, E. R., Folt, C. L., Goldman, C. R., 1984. Copepod and cladoceran success in an oligotrophic lake. Journal of plankton research, 6(1), 45-65.

C --

Cardoso, P.G., Marques, S.C., D'Ambrosio, M., Pereira, E., Duarte, A.C., Azeiteiro, U.M., Pardal, M.A., 2013. Changes in zooplankton communities along a mercury contamination gradient in a coastal lagoon (Ria de Aveiro, Portugal). Marine Pollution Bulletin 76(1-2), 170-177.

Counahan, D.F., Carline, R.F., Reid, J.W., 2005. The occurrence of the calanoid copepod *Eurytemora affinis* (Poppe) in the upper Ohio River. Northeastern Naturalist 12, 541-545.

Cox, T.J.S., Maris, T., Soetaert, K., Conley, D.J., Van Damme, S., Meire, P., Middelburg, J.J., Vos, M., Struyf, E., 2009. A macro-tidal freshwater ecosystem recovering from hypereutrophication: the Schelde case study. Biogeosciences 6, 2935-2948.

D --

David, V., Sautour, B., Chardy, P., Leconte, M., 2005. Long-term changes of the zooplankton variability in a turbid environment: the Gironde estuary (France). Estuarine, Coastal and Shelf Science, 64(2), 171-184.

De Pauw, N., 1975. Bijdrage tot de kennis van milieu en plankton in het Westerschelde estuarium. PhD, thesis University of Ghent (Belgium). I (in Dutch).

Devreker, D., Souissi, S., Winkler, G., Forget-Leray, J., Leboulenger, F., 2009. Effects of salinity, temperature and individual variability on the reproduction of *Eurytemora affinis* (Copepoda; Calanoida) from the Seine estuary: a laboratory study. Journal of Experimental Marine Biology and Ecology, 368(2), 113-123.

Dobberfuhl, D.R., Miller, R., Elser, J.J., 1997. Effects of a cyclopoid copepod (*Diacyclops thomasi*) on phytoplankton and the microbial food web. Aquatic Microbial Ecology 12, 29-37.

Ducrotoy, J.P., Dauvin, J.C., 2008. Estuarine conservation and restoration: the Somme and the Seine case studies. Marine Pollution Bulletin 57, 208-218.

Druschke, C.G., Hychka, K., 2015. Manager perspectives on communication and public engagement in ecological restoration project success. Ecology and Society 20(1), 58.

E --

Etcheber, H., Schmidt, S., Sottolichio, A., Maneux, E., Chabaux, G., Escalier, J.M., Wennekes, H., Derriennic, H., Schmeltz, M., Quemener, L., Repecaud, M., Woerther, P., Castaing, P., 2011. Monitoring water quality in estuarine environments: lessons from the MAGEST monitoring program in the Gironde fluvial-estuarine system. Hydrology and Earth System Science, 15(3), 831-840.

Falcao, J., Marques, S.C., Pardal, M.A., Marques, J.C., Primo, A.L., Azeiteiro, U.M., 2012. Mesozooplankton structural responses in a shallow temperate estuary following restoration measures. Estuarine, Coastal and Shelf Science 112, 23-30.

Feike, M., Heerkloss, R., 2008. Long-term stability of the seasonal succession of different zooplankton species in a brackish water lagoon (southern Baltic Sea). Hydrobiologia, 611(1), 17-28.

Flaherty, K., Matheson, R.E., McMichael, R.H., Perry, W.B., 2013. The influence of freshwater on nekton community structure in hydrologically distinct basins in Northeastern florida bay, fl, USA. Estuaries and Coasts 36, 918-939.

Fox, H.M., 1957. Haemoglobin in crustacea. Nature 179, 148.

G --

Gasparini, S., Castel, J., Irigoien, X., 1999. Impact of suspended particulate matter on egg production of the estuarine copepod, *Eurytemora affinis*. Journal of Marine Systems, 22(2), 195-205.

Green, J., 1959. Haemoglobin and the habitat of the harpacticoid copepod Elaphoidella gracilis (Sars). Nature 27, 1834.

Grosjean, P., Picheral, M., Warembourg, C., Gorsky, G., 2004. Enumeration, measurement, and identification of net zooplankton samples using the ZOOSCAN digital imaging system. ICES Journal of Marine Science 61(4), 518-525.

Güde, H., 1989. The Role of Grazing on Bacteria in Plankton Succession. In Sommer, U., Plankton ecology. Succession in plankton Communities. Springer-Verlag, Berlin, 337-358pp.

Guisan, A., & Zimmermann, N. E., 2000. Predictive habitat distribution models in ecology. Ecological modelling, 135(2), 147-186.

H --

Hammock, B. G., Lesmeister, S., Flores, I., Bradburd, G. S., Hammock, F. H., Teh, S. J., 2016. Low Food Availability Narrows the Tolerance of the Copepod *Eurytemora affinis* to Salinity, but Not to Temperature. Estuaries and Coasts, 39(1), 189-200.

Hawkins, S.J., Gibbs, P.E., Pope, N.D., Burt, G.R., Chesman, B.S., Bray, S., Proud, S.V., Spence, S.K., Southward, A.J., Langston, W.J., 2002. Recovery of

polluted ecosystems: the case for long-term studies. Marine Environmental Research 54, 215-222.

Heip, C., 1988. Biota and abiotic environmrnt in the Westerschelde estuary. Aquatic Ecology 22, 31-34.

Heinle, D.R., Flemer, D.A., 1975. Carbon requirements of a population of the estuarine copepod *Eurytemora affinis*. Marine Biology 31(3), 235-247.

Hogg, R.S., Coghlan, S.M., Zydlewski, J., Gardner, C., 2014. Fish community response to small-stream dam removal in a marine coastal river tributary. Transactions of the American Fisheries Society 144(3), 467-479.

K--

Kemp, W.N, Boynton, W.R., Adolf, J.E., Boesch, D.F, Boicourt, W.C., Brush, G., Cornwell, J.C., Fisher, T.R., Glibert, P.M., Hagy, J.D., Harding, L.W., Houde, E.D., Kimmel, D.G., Mille, W.D., Newell, R.I.E., Roman, M.R., Smith, E.M., Stevenson, J.C., 2005. Eutrophication of chesapeake bay: historical trends and ecological interactions. Marine Ecology Progress Series 303, 1-29.

Kimmel, D.G., Roman, M.R., Zhang, X., 2006. Spatial and temporal variability in factors affecting mesozooplankton dynamics in Chesapeake Bay: Evidence from biomass size spectra. Limnology and Oceanography 51(1), 131-141.

L --

Langseth, B.J., Purcell, K.M., Craig, J.K., Schueller, A.M., Smith, J.W., Shertzer, K.W., Creekmore, S., Rose, K.A., Fennel, K., 2014. Effect of changes in dissolved oxygen concentrations on the spatial dynamics of the Gulf Menhaden fishery in the northern Gulf of Mexico. Marine and Coastal fisheries: Dynamics, Management, and Ecosystem Science 6(1), 223-234.

Lankov, A., Ojaveer, H., Simm, M., Pollupuu, M., Möllmann, C., 2010. Feeding ecology of pelagic fish species in the Gulf of Riga (Baltic Sea): the importance of changes in the zooplankton community. Journal of Fish Biology 17(10), 2268- 2284.

Lee, C.E., Bell, M. A., 1999. Causes and consequences of recent freshwater invasions by saltwater animals, Trends in Ecology & Evolution 14(1), 284-288.

Lee, C.E., Rempert, J.L., Gelembiuk, G.W., 2003. Evolution of physiological tolerance and performance during freshwater invasions. Integrative &. Compartive Biology 43(3), 439-449.

Lee, C.E., Posavi, M., Charmantier, G., 2012. Rapid evolution of body fluid regulation following independent invasions into freshwater habitats. Journal of Evolutionary Biology 25(4), 625-633.

Lee C.E, Moss, W.E., Olson, N., Chau, K.F., Chang, Y.M., Johnson, K.E., 2013. Feasting in freshwater: impacts of food concentration on freshwater tolerance and the evolution of food x salinity response during the expansion from saline into freshwater habitats. Evolutionary Applications 6(4), 673-689.

Lelièvre, S., Antajan, E., Vaz, S., 2012. Comparison of traditional microscopy and digitized image analysis to identify and delineate pelagic fish egg spatial distribution. Journal of Plankton Research 34 (6), 470-483.

M --

Maes, J., Tackx, M., Soetaert, K., 2005. The predation impact of juvenile herring *Clupea harengus* and sprat *Sprattus sprattus* on estuarine zooplankton. Hydrobiologia 540, 225-235.

Marcolin, C.R., Schultes, S., Jackson, G.A., Lopes, R.M., 2013. Plankton and secton size spectra estimated by the LOPC and Zooscan in the Abrolhos bank ecosystem (SE atlantic). Continental Shelf Research 70(1), 74-87.

Martino, E.J., Houde, E.D., 2010. Recruitment of striped bass in Chesapeake Bay: spatial and temporal environmental variability and availability of zooplankton prey. Marine Ecology Progress Series 409, 213-228.

Mehner, T, 2011. Diet spectra of *Pomatoschistus microps* (Kroyer) and *Pomatoschistus minutus* (Pallas) (Teleostei, gobidae) during 1st week after hatching. Zoologischer Anzeiger 229, 13-20.

Maire A., Buisson L., Biau, S., Canal, J., Lafaille, P., 2013. A multi-faceted framework of diversity for prioritizing the conservation of fish assemblages. Ecological Indicators 34, 450-459.

Meire, P., Ysebaert, T., Van Damme, S., Van den Bergh, E., Maris, T., Struyf, E., 2005. The Scheldt estuary: a description of a changing ecosystem. Hydrobiologia 540(1), 1-11.

Mialet, B., Azémar, F., Maris, T., Sossou, C., Ruiz, P., Lionard, M., Van Damme, S., Lecerf, A., Muylaert, K., Toumi, N., Tackx, M., 2010. Spatial spring distribution of the copepod *Eurytemora affinis* (Copepoda, Calanoida) in a restoring estuary, the Scheldt (Belgium), Estuarine, Coastal and Shelf Science, 88, 116-124.

Mialet, B., Gouzou, J., Azémar, F., Maris, T., Sossou, C., Toumi, N., Van Damme, S., Meire, P., Tackx, M., 2011. Response of zooplankton to improving water quality in the Scheldt estuary (Belgium). Estuarine, Coastal and Shelf Science 93, 47-57.

N, O --

Nobriga, M.L., 2002. Larval delta smelt diet composition and feeding indice: Environmental ant ontogenetic influences. California fish and game 88, 149-164pp.

Ooms-Wilms, A.L., Postema, G., Gulati, R.D., 1995. Evaluation of bacterivory of rotifera based on measurements of in-situ ingestion of fluorescent particles, including some comparisons with cladocera. Journal of Plankton Research 17(5), 1057-1077.

Orson, R.A., Howes, B.L., 1992. Salt Marsh development studies at Waquoit Bay, Massachusetts: influence of geomorphology on long-term plant community structure. Estuarine, Coastal and Shelf Science 35(5), 453-471.

Ostrensky, A., Marchiori, M. A., Poersch, L. H., 1992. [Acute toxicity of ammonia in the production process of postlarvae of Penaeus paulensis Perez-Farfante, 1967]. Anais da Academia Brasileira de Ciências, 64(4), 383-389.

P, R--

Pirow, R., Bäumer, C., Paul, R.J., 2001. Benefits of haemoglobin inn the cladoceran crustacean Daphnia magna. The Journal of Experimental Biology 204, 3425-3441.

Roman, M.R., Rublee, P.A., 1980. Containement effect s in copepod grazing experiments: A plea to end the black box approach. Limnology and Oceanography 25, 982-990.

Romero, E., Le Gendre, R., Garnier, J., Billen, G., Fisson, C., Silvestre, M., Riou, P., 2016. Long-term water quality in the lower Seine: Lessons learned over 4 decades of monitoring. Environmental Science & Policy, 58, 141-154.

S --

Sanders, R.W., Porter, K.G., Bennett, S.J., DeBiase, A.E., 1989. Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnology and Oceanography 34(4), 673-687.

Sautour B., Castel, J., 1995. Spring zooplankton distribution and production of the copepod Euterpina acutifrons in Marennes-Oléron Bay (France). Hydrobiologia 310, 163-175.

Sautour B., Castel, J., 1995. Comparative spring distribution of zooplankton in three macrotidal European estuaries. Hydrobiologia 311, 139-151.

Sell, A.F., 1998. Adaptation to oxygen deficiency: Contrasting patterns of haemoglobin synthesis in two coexisting *Daphnia species.* Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 120(1), 119-125.

Sell, A., 2000. Life in the extreme environment at a hydrothermal vent: haemoglobin in a deep-sea copepod. Proceedings of the Royal Society: B-Biological Sciences 267, 2323-2326.

Schultes, S., Lopes, R.M., 2009. Laser optical plankton counter and zooscan intercomparison in tropical and subtropical marine ecosystems. Limnology and oceanography methods 7(11), 771-784.

Smit, H., Smits, R., Van der Velde, G., Coops, H. 1997. Ecosystem responses in the Rhine-Meuse delta during two decades after enclosure and steps toward estuary restauration. Estuaries 20(3), 504-520.

T --

Tackx, M., De Pauw, N., Van Mieghem, R., Azémar, F., Hannouti, A., Van Damme, S., Fiers, S., Daro, N., Meire, P., 2004. Zooplankton in the Schelde estuary, Belgium and the Netherlands. Spatial and temporal patterns. Journal of Plankton Research 26, 133-141.

Ter Braak, C. J. F., 1987. Ordination. In Jongman, R. H. G., C. J. F. ter Braack & O. F. R. Van Tongeren (eds.), Data analysis in community and landscape ecology. Pudoc, Wageningen, Netherlands, 91-173.

Ter Braak, C. J. F., 1994. Canonical community ordination. Part I: basic theory and linear methods. Ecoscience 1,127-140.

Teuchies, J., Singh, G., Bervoets, L., Meire, P., 2013. Land use changes and metal mobility: Multi-approach study on tidal marsh restoration in a contaminated estuary. Science of the Total Environment 449, 174-183.

V, W – –

Van Damme S., Struyf, E., Maris, T., Ysebaert, T., Dehairs, F., Tackx, M., Meire, P., 2005. Spatial and temporal patters of water quality along the estuarine salinity gradient of the Scheldt estuary (Belgium and The Netherlands): results of an integrated monitoring approach. Hydrobiologia 540, 29 -45.

Vad, CF., Horyvath, Z., Kiss, K.T., Acs, E., 2012. Microcrustacean (cladocera, copepoda) communities in artificial lakes in the region of the North Hungarian mountains with special reference to adventive species. Acta Zoologica Academiae Scientiarum Hungaricae 58, 57-61.

Van den Meersche, K., Van Rijswijk, P., Soetaert, K., Middelburg, J.J., 2009. Autochthonous and allochthonous contribution to mesozooplankton diet in a tidal river and estuary: Integrating carbon isotope and fatty acid constraints. Limnology and Oceanography 54(1), 62-74.

Verissomo, H., Larie, M., Patricio, J., Gamito, S., Marques, J.C., 2013. Trends in water quality and subtidal benthic communities in a temperate estuary: Is the response to restoration efforts hidden by climate variability and the Estuarine Quality paradox? Ecological Indicators 24, 56-67.

Verity, P.G., 2002a. A decade of change in the Skidaway river estuary. I. Hydrography and nutrients. Estuaries 25(5), 944-960.

Verity, P.G., 2002b. A decade of change in the Skidaway river estuary. II. Particulate organic carbon, nitrogen and chlorophyll *a*. Estuaries 25(5), 961-975.

Verity, P.G., Borkman, D.G., 2010. A decade of change in the Skidaway river estuary. III. Plankton. Estuaries and Coasts 33, 513-540.

Viitasalo, M., Rautio, M., 1998. Zooplanktivory by *Praunus flexuosus* (Crustacea, Mysidacea): functional responses and prey selection u in relation to prey escape responses. Marine Ecology Progress Series 174, 77-87.

Walsh, C. J., MacNally, R., 2004. The hier. part package version 1.0 Hierarchical Partitioning.

III. CHAPITRE 3 :

Tolérance du zooplancton estuarien à de faibles concentrations en oxygène

III.1 Résumé

III.1.1 Contexte et objectifs

Comme nous avons pu démontrer dans le premier chapitre, d'importants changements de la qualité de l'eau sont survenus dans l'estuaire de l'Escaut (Van Damme et al., 2005 ; Cox et al., 2009 ; Mialet et al., 2011). De plus, nous avons constaté l'enrichissement en oxygène dans la partie amont de l'Escaut. Entre 1996 et 2006, la communauté zooplanctonique était dominée par les copépodes cyclopoïdes et les cladocères. Depuis 2007, un changement communautaire a eu lieu, le copépode calanoïde *E. affinis* s'y est fortement développé au détriment des cyclopoïdes. Issu de ce constat un intérêt particulier est porté sur ce copépode : *E. affinis*. Ce copépode printanier domine une grande partie des zones d'eau saumâtre de l'hémisphère Nord (Lee, 1999). Les travaux d'Appeltans et al. (2003) suggèrent un effet seuil de la concentration en oxygène dissous sur la présence de ce copépode. Les auteurs émettent l'hypothèse que, dans l'Escaut, les conditions hypoxiques aux alentours d'Anvers agiraient comme une barrière écologique, empêchant les populations de ce copépode de migrer d'aval en amont. Depuis 2007 (chap.1), on observe l'espèce de façon permanente et en grande abondance dans le tronçon d'eau douce en amont de l'Escaut.

L'objectif de cette étude a été de tester expérimentalement la tolérance aux faibles concentrations d'oxygène, pour *E. affinis* mais aussi pour les copépodes cyclopoïdes. Pour cela les espèces zooplanctoniques ont été incubées dans de l'eau naturelle durant 2 à 24h et selon un gradient de concentration en oxygène dissous. A chaque échantillon, les organismes morts ont été comptés sous binoculaire et la totalité de l'échantillona été fixé au formol afin d'être conservé pour le comptage de l'abondance totale.

III.1.2 Principaux résultats

Entre 2 et 4,5 h d'incubation les taux de mortalités des calanoïdes et les cladocères ont diminué avec l'augmentation de la concentration en oxygène entre 0,5 et 7,22 mg L^{-1}. Le taux de mortalité des cyclopoides ne variait pas avec la concentration en oxygène et, était inférieure à celle des calanoïdes et des cladocères.

Les conséquences de cette différence de tolérance pour une faible concentration d'oxygène entre les taxa de zooplancton sont discutées en relation avec le développement des communautés de zooplancton dans l'Escaut, et des estuaires en général.

Nos résultats sur la tolérance à faible concentration d'oxygène de trois ordres différents de zooplancton, combinées à l'évolution de la communauté de zooplancton dans l'estuaire de l'Escaut suivantes amélioration de la qualité de l'eau et l'augmentation de la concentration en oxygène associée, montrent clairement que la tolérance à l'hypoxie est un facteur important dans la détermination de la composition des communautés zooplanctoniques dans l'eau douce de l'Escaut.

Le développement d'*E. affinis* dans la zone amont n'a été possible que depuis l'amélioration de la qualité de l'eau et donc l'augmentation de la concentration en oxygène dissous. Une fois cette condition réalisée *E. affinis* pouvait se développer en amont, il est rapidement devenu une espèce dominante, comme observé dans plusieurs autres systèmes (Counahan et al 2005; Vad et al 2012). Davidson et al. (2000), avec leur étude sur les communautés de zooplancton dans divers habitats des plaines inondables de la rivière Atchafalaya (USA), ont aussi observéune forte dominance de quelques taxa zooplanctoniques sur des sites bien oxygénés, par rapport à des sitesplus riches en détritus, où l'abondance du zooplancton était plus faible, mais plus diversifiée et représentant uniformément divers taxons. Ainsi, il peut être envisagé que la dominance de *E. affinis* dans l'Escaut en amont n'est peut-être pas typique pour cette espèce, mais illustre un modèle plus général de la diversité de la communauté par rapport aux conditions environnementales.

III.2 Article 2 : Estuarine zooplankton tolerance for low oxygen concentration

Estuarine zooplankton tolerance for low oxygen concentration

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Manuscript in preparation

III.2.1 Abstract

Water quality in the Scheldt estuary (Belgium/The Netherlands) has improved since two decades. Oxygen concentration has increased, especially in the upstream freshwater tidal reach. The zooplankton community in this area was dominated by cyclopoid copepods and cladocerans between 1996 and 2006. Since 2007, a change in community composition occurred: the calanoid copepod *E. affinis* developed strongly, and cyclopoid copepods decreased in abundance.

The present study experimentally tested the tolerance of calanoid copepods (in casu *E. affinis*) and cyclopoid copepods for low oxygen concentrations.
Zooplankton was incubated in the laboratory in Scheldt water gradient of oxygen concentration from 0.5 to 7.22 mg L^{-1} during 2 to 24 h. At each sampling, dead organisms were counted under binocular microscope and the total sample was fixed with formalin afterwards for counting of total abundance.

Between 2 and 4,5 h of incubation calanoid and cladoceran mortality rates decreased with increasing oxygen concentration between 0.5 and 7.22 mg L^{-1}. Cyclopoid morality rate did not vary with oxygen concentration and was lower than that of calanoids and cladocerans.

The consequences of this difference in tolerance for low oxygen concentration between zooplankton orders is discussed in relation to zooplankton community development in the Scheldt, and estuaries in general.

Key words:
Low oxygen, estuary, zooplankton, *Eurytemora affinis,* cyclopoid copepods.

III.2.2 Introduction

As an essential element to most metazoans, oxygen concentration in aquatic systems has an important influence on the composition of communities and their spatio-temporal distribution. In both marine and freshwater systems, low oxygen concentrations can occur either by natural causes or by eutrophication. Concerns about the influence of hypoxia on organisms, their distribution and the resulting functioning of the ecosystem lead to many studies, but these are mainly focused on fish and large – sized crustaceans (e.g. Wu et al., 2002; Moyson et al., 2004; Greg et al, 2014; Bonvillain et al., 2015). Research on the response of zooplankton to hypoxia concerns mainly coastal shelf regions and estuarine bays, in which distinct low oxygen concentration strata develop during at least a part of the year such as, for instance, the coast of central Peru, Puget Sound (USA) or Chesapeake Bay (USA) (Criales-Hérnandez et al., 2008; Elliot et al., 2013; Keister and Tuttle, 2013). Among the dominant taxa of such systems, the calanoid copepod *Acartia tonsa* is one of the most studied in relation to its hypoxia tolerance and vertical positioning in relation to the oxycline. Field observations have shown that summer hypoxia (generally considered as < 2 mg L^{-1}) in the lower part of the water column negatively affects the abundance of this species. Both laboratory experiments and field data indicate that, while hypoxia seems to increase mortality of *A. tonsa* nauplii, its effect on copepodites seems sublethal. Nevertheless, anoxia does have a negative effect on *A. tonsa* abundance, either through increased exposure to predation following vertical anoxia avoidance, or through physiologic weakening caused by increased energy requirements, resulting in lower somatic growth and egg production (Marcus et al., 2004, 2005, Richmond at al., 2006; Elliot et al., 2013a, b).

Studying the northern Gulf of Mexico zooplankton community structure in relation to environmental conditions, including summer hypoxia, Elliot et al. (2012) found a differentiation in hypoxia effects on the abundance of taxa. One group of taxa composed of the calanoid copepods *Acartia* spp., cladocerans, copepod nauplii, harpacticoids and gelatinous medusa was most abundant at high temperature (29–32°C), low to intermediate salinities (12–33) and conditions with less than 30 % of the water column being hypoxic. Another group of dominant taxa, composed of the calanoid copepods *Centropages* spp., *Temora* spp., *Paracalanus* spp., the cyclopoid copepods *Oithona* spp and *Corycaeus* spp. and salps were associated with higher

salinities (>27) and temperatures between 28-31°C and were most abundant when 40 % of the water column was hypoxic. Looking at the effect of hypoxia on the total zooplankton community of a sub-estuary of Puget Sound, Keister and Tuttle (2013) showed that oxygen concentration in this system does not play a major role in structuring the community taxonomic composition, but influences the vertical positioning in the water column of several species. Meroplanktonic organisms, larvae and the copepod *Paracalanus parvus* were generally found above the oxycline, while polychaetes and the calanoid copepod *Metrida pussilus* occurred below the oxycline, and the vertical positioning of the cyclopoid *Oithona similis* showed no relation to the oxycline. So, while the literature on marine and estuarine zooplankton tolerance and response to anoxia suggest that the response of zooplankters is taxa specific, and can vary according to the type of ecosystem, suggesting the capacity for low oxygen concentration avoidance and tolerance of sub-lethal concentration for several copepod species, at least from the copepodite stage onwards.

Hypoxia being wide spread in freshwater systems, the tolerance for hypoxia has been quite extensively studied for cladocerans as they occur in many low oxygenated systems and often during warm, hypoxic periods. All branchiopoda have hemoglobin (Terwiller and Ryan, 2001) and many cladocerans (e.g. *Daphnia, Bosmina, Chidorus, Simocephalus, Moinia*) are known as tolerant to rather severe hypoxia, because of their capacity to adjust the level of hemoglobin (Hb) in the haemolymphe (e.g. Fox, 1948, 1957; Svetlichny et Hubareva, 2002; Pirow et Buchen, 2004). However, little information is available about Hb in copepods. To the best of our knowledge, it has only been reported in a few parasites (Fox, 1957) and mud dwelling and borrow habiting harpacticoids (Fox, 1957; Green, 1959) and in some taxa from hydrovent environments (Hourdez et al., 2000; Sell, 2000).

The present study was inspired by changes occurring in zooplankton community composition in the freshwater part of an estuary, harboring both cladocerans and copepods as main mesozooplankters. The Scheldt estuary (Belgium/ The Netherlands) covers a total salinity gradient including a 60 km freshwater stretch under tidal influence (Fig. 1) (Meire et al., 2005; Van Damme et al., 2005). During the second half of the 20th century, the Scheldt was heavily polluted. Besides chemical pollution, organic matter and nutrients represented the main pollution arising from discharge within the heavily habituated and cultured drainage basin (Baeyens et al.,

1998; Van Damme et al., 2005). This pollution led to very low oxygen concentrations, mainly in the freshwater area between km 95 – km 164. (km 90; Fig. 21). Oxygen saturation levels in the surface water were lower than 40 % during the major part of the year. Since the 1980ties measures were taken to decrease pollution in the catchment and the Scheldt has been recovering.

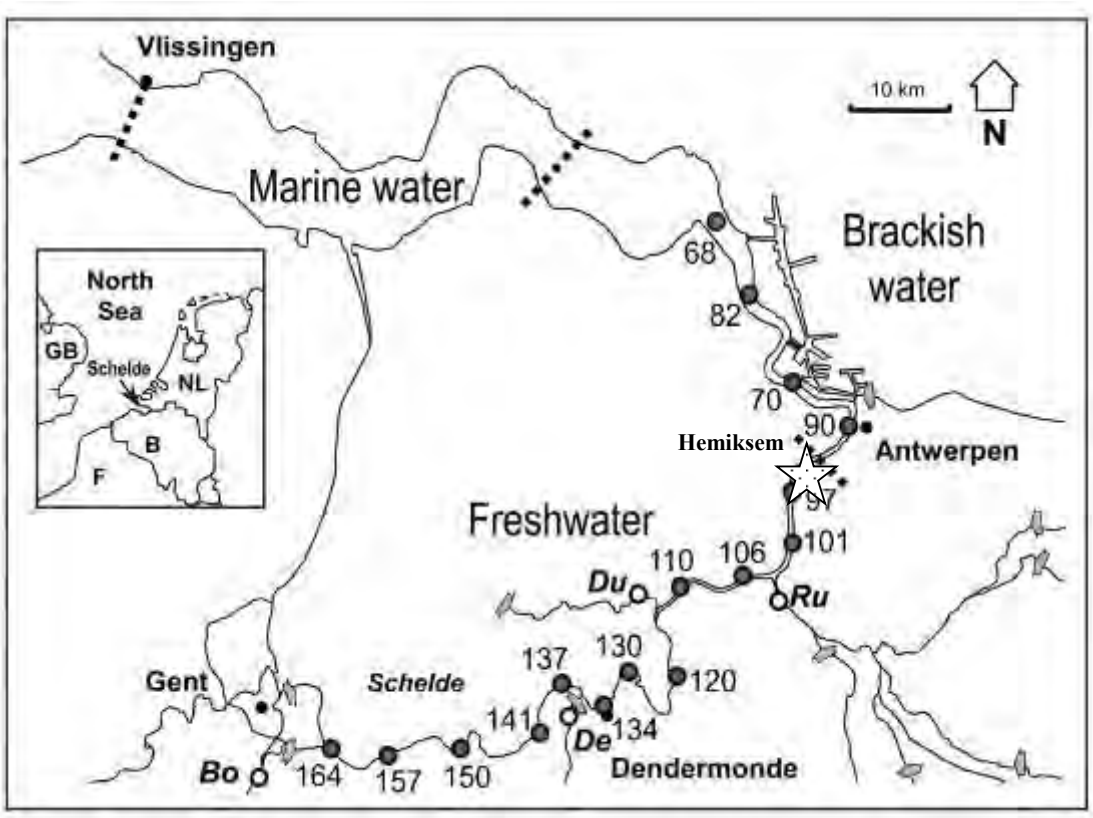

Figure 21 : Map of the Scheldt estuary with OMES sampling stations, designated by their distance, in km, upstream from Vlissingen (mouth). Dotted lines indicate limits between marine water, brackish water and freshwater reaches. Hemiksem station considered in this study is represented by the star.

Between 2000 and 2003, the upstream Scheldt gradually changed from hypereutrophic, heterotrophic system to eutrophic, autotrophic system (Cox et al., 2009). Lower organic and nutrient loads led to an improving oxygen concentration and strong phytoplankton primary production, which was, prior to the switch, hampered by too low oxygen concentrations or high ammonia concentrations. The substantial increase in oxygen concentration in the freshwater reach, co-occurred with a decrease in NH_4 and NO_3 concentration, and an initially strong increase in chlorophyll concentration. Detailed descriptions of the evolution of the Scheldt system can be found in Meire et al. (2005), Van Damme et al. (2005), Cox et al. (2009), Mialet et al. (2011).

Earlier studies have shown the freshwater area of the Scheldt to be very poor in zooplankton and hyperbenthos (Soetaert et Van Rijswijk, 1993). A comparison of the spatial distribution of *E. affinis* in the Ems (The Netherlands), the Gironde (France) and the Scheldt estuary, showed that in the two former estuaries, the dominant copepod *E. affinis* abundance peaked between 0 and 6 salinity, while in the Scheldt its maximum abundance was observed between 10–12 salinity. This 'displacement' was attributed to the very low oxygen concentration in the 0–2 salinity zone of the Scheldt (km 70–160) compared to the other two estuaries, where this salinity range apparently corresponded to the optimum for *E. affinis* (Soetaert et Van Rijswijk, 1993; Sautour et Castel, 1995). Appeltans et al. (2003), reported that the Scheldt spring *E. affinis* population reached higher abundance (320–14 000 ind. m^{-3}) in the 0–2 salinity zone during 1996–'97 compared to 1989–'90, when its abundance was between 0 and 160 ind. m^{-3}. This increase was explained by the improved water quality and higher spring oxygen concentrations in this area (3.52–7.05 mg L^{-1}). In the following years, *E. affinis* was sporadically observed in the freshwater stretch in variable abundances. Mialet et al. (2010), demonstrated that the presence and abundance of *E. affinis* in the Scheldt freshwater stretch during the 1996–2007 was related to the oxygen concentration in the upstream Scheldt. *E. affnis* could be abundant upstream if the oxygen concentration there was > 4 mg L^{-1}. Mialet et al. (2011) reported a remarkable change in the zooplankton community in parallel with water quality improvement. Between 1996 and 2006, the brackish water stretch was dominated by calanoid copepods, with *E. affinis* as dominant species, mainly occurring in spring. The freshwater zooplankton community was more diverse, with a dominance of several cyclopoid copepod species and cladocerans, and few calanoid copepods (Tackx et al., 2004; Mialet et al., 2011). From 2007 onwards, a strong development of *E. affinis* took place in the freshwater stretch. At the same time, cyclopoid copepods decreased strongly in abundance, which resulted in a 50–90 % *E. affinis* contribution to the freshwater copepod community, whereas before, it represented maximally 25 %. Cladoceran average yearly abundance remained unchanged over time (Mialet et al., 2011; Chambord et al., accepted).

Considering that on the one hand, cyclopoids and cladocerans were abundant in the upstream freshwater stretch of the Scheldt estuary prior to water quality improvement, and on the other hand, there was a clear association between oxygen

concentration and *E. affinis*' abundance in this zone, we formulated the hypothesis that cyclopoids and cladocerans have a higher tolerance for low oxygen concentration than the calanoid *E.* affinis. This hypothesis was tested by exposing concentrated natural Scheldt zooplankton communities to a gradient of oxygen concentrations and observing the mortality of the different orders of zooplankters. Evidently, besides oxygen concentration, other environmental variables (e.g. NH_4 concentration, pH…) changed in parallel in the Scheldt estuary. However, multivariate analysis has shown oxygen concentration to be a main driver of changes occurring in the zooplankton community (Chambord et al., accepted, chapter II) and hence, its specific influence was experimentally tested.

III.2.3 Material and methods

The Scheldt has its source in the North of France and runs through Belgium to join the North Sea at Vlissingen in the Netherlands. Its estuary is situated from the mouth at Vlissingen until the city of Gent, where the tide is stopped by sluices (Fig. 21). Contrarily to most of the other temperate estuaries, the Scheldt estuary is characterized by vertically well mixed water flows (Baeyens et al., 1998), inducing most of the time no salinity or current stratification (Heip, 1988).

50 L of Scheldt water was collected in Hemiksem (51°08'35.49"N; 4°19'48.56"E) situated in the freshwater tidal area of the estuary (Fig 21). At the same occasion, zooplankton was collected by submerging a 50 µm net counter current for 10 to 30 minutes according to the prevailing current. The collected zooplankton was transferred to a 1 L container and water and zooplankton were transported to the laboratory. In the laboratory, series of 1 L bottles were filled up till 970 mL with Scheldt water, filtered on a 50 µm mesh. These bottles were bubbled with nitrogen during various periods to obtain a gradient in oxygen concentration. Per oxygen concentration, 5 replicate bottles were used for incubation during 2, 4, 6, 18, and 24h. A first series of 4 experiments was realized between 2 and 5 April 2014 and a second series of 5 experiments between 9 and 13 July 2014. The 9 experiments covered an oxygen concentration range between 1 and 8 mg L^{-1}. Within one experiment, the difference between minimum and maximum oxygen concentration was between 0.5 and 2 mg L^{-1}.

The zooplankton was concentrated over a 50 µm mesh, and 30 mL aliquots were added to each experimental bottle at t_0 of the experiment. Oxygen concentration was measured in each bottle with an oxygen sensor (model: WTW Oxi323 with a WTW Cellox325 sensor) at t_0 and t_{end} of the experiment.

At each incubation time, the zooplankton of 5 bottles was separately collected with a 50 µm mesh and concentrated in 60 mL. From these concentrates, 6 mL subsamples were analyzed under binocular microscope to count the dead individuals, distinguishing adults of calanoids, cyclopoids and cladocerans. Afterwards, the 6 mL subsamples were fixed with formalin (4 % final concentration) for subsequent counting of the total abundance of each zooplankton order under binocular microscope.

Mortality was calculated as the number of dead organisms of each group observed at each sampling, expressed as percentage of the total abundance of each group in each bottle. Mortality rate was calculated as mortality per hour, also expressed in percentage of total abundance. Oxygen concentration on axes in the graphs are mean values between t_0 and t_{end} in each experimental bottle.

III.2.4 Results

III.2.4.1 Incubation time

The mortality of calanoids, cyclopoids and cladocerans did not show any tendency with incubation time as can be seen from two examples representing an experiment in the low oxygen concentration range (1,6 – 2,7 mg L^{-1}) and one in a higher oxygen concentration range (3,6 – 4,9 mg L^{-1}) (Fig. 22a, b). So, independent of the oxygen concentration, the mortality occurring at the longer incubation times had already occurred earlier.

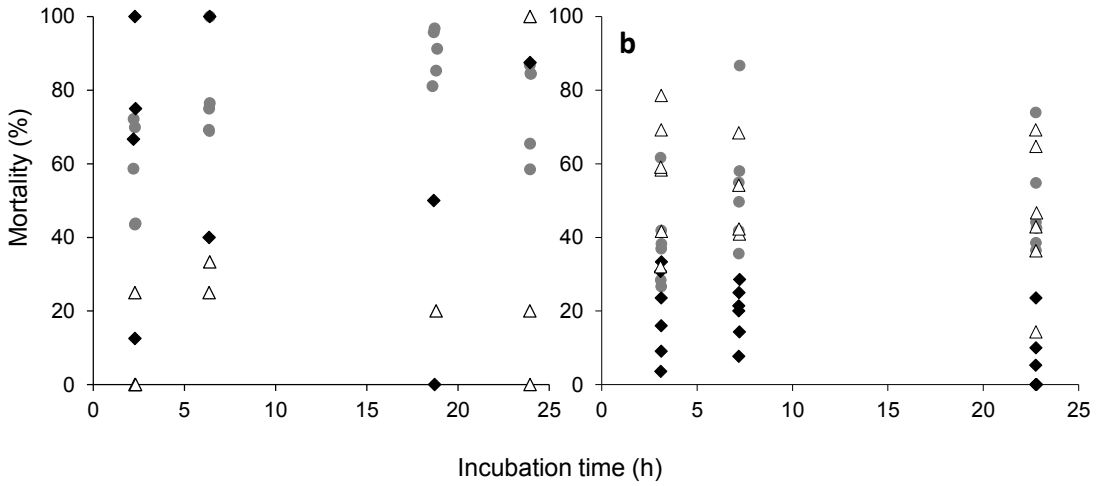

Figure 22 : Examples of mortality obtained for calanoids (grey circles), cyclopoids (black diamonds) and cladocerans (white triangles) as a function of incubation time. a) experiment run at 1,6-2,7 mg L^{-1} and b) experiment run at 3,6-4,9 mg L^{-1} oxygen concentration.

Consequently, for further analysis, only the results obtained at incubation times between 2 and 6 h were considered.

III.2.4.2 Density of organism

Because in 2014, the Scheldt freshwater zooplankton community was already strongly dominated by *E. affinis*, we had to substantially concentrate the natural zooplankton in order to have a sufficient number of observations on cyclopoids and cladocerans in each experimental bottle. Bottles in which any of these were below 4 ind. L^{-1} have been discarded from the analysis. The number of individuals observed (dead and alive) varied between 4–50 cyclopoids, 15–156 calanoids and 4–81 cladocerans. The high abundance of total individuals incubated might have led to stressful experimental conditions. To verify that this potential stress was not the main driver of mortality, we analyzed mortality rates for each order as a function of the number of individuals incubated, considering the three orders together. No trend between the number of individuals incubated and mortality rate was found for any of the orders (Fig. 23).

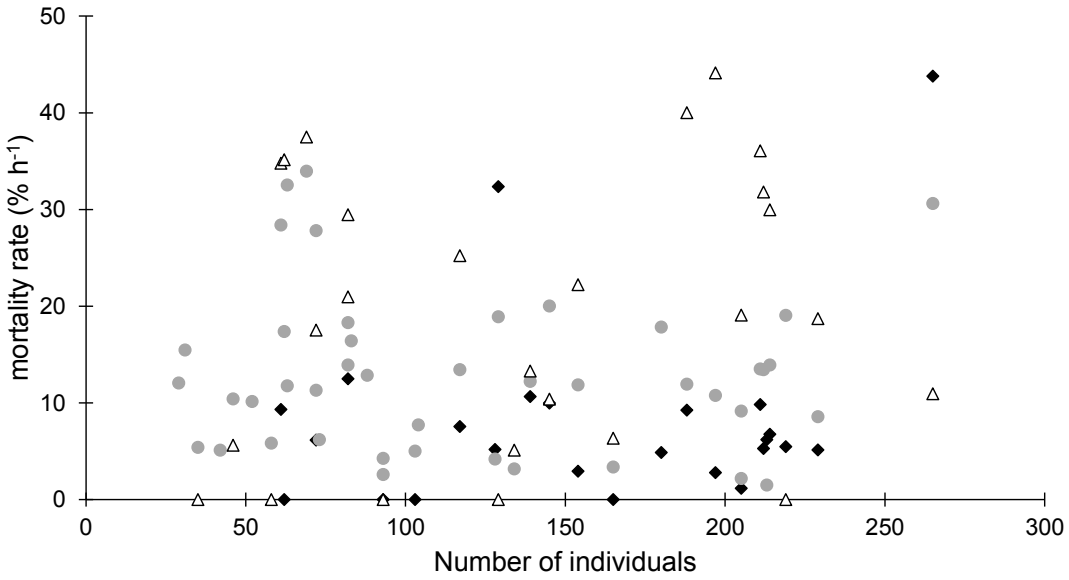

Figure 23 : Mortality rate of calanoids (grey circles) cyclopoids (black diamonds) and cladocerans (white triangles) as a function of number of individuals incubated, considering the three groups together.

Morality rates of both calanoids and cladocerans decreased significantly (spearman rank, p= 6.78e-07 and p= 0.00018 respectively) with oxygen concentration (Fig 24).The mean mortality rate observed within the 0.5–7.22 mg L^{-1} oxygen concentration range covered by the experiments did not differ significantly between calanoids and cladocerans (Mann Whitney, p= 0.1011). Cyclopoid mortality rates, on the contrary, did not vary significantly with oxygen concentration (spearman rank, p= 0.191), and were significantly lower than those of calanoids and cladocerans (Mann Whitney, p= 0.00094 and p= 0.00812 respectively) (Fig. 24).

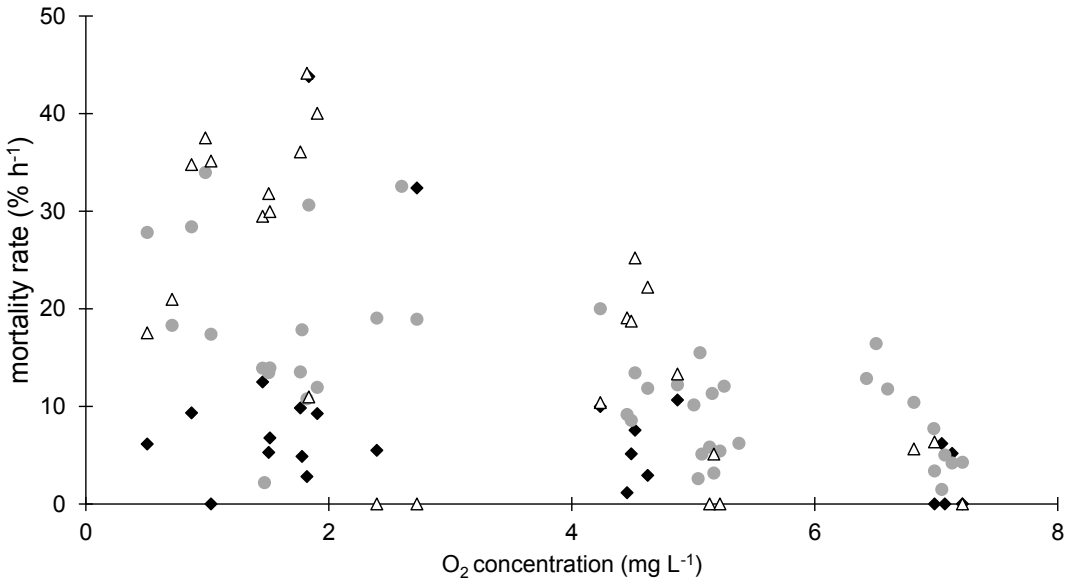

Figure 24 : Mortality rate of calanoids (grey circles) cyclopoids (black diamonds) and cladocerans (white triangles) as a function of oxygen concentration. Oxygen concentrations on x axis are mean values between t_0 and t_{end} for each experimental bottle.

III.2.5 Discussion

We used visual observation of dead animals rather than staining with dyes. Several authors have successfully used neutral red in zooplankton studies in estuarine, calanoid dominated systems (e.g; Tang et al., 2006; Kimmel et al., 2009a, b). Bickel et al. (2009) stained dead copepods and cladocerans from freshwater lakes with satisfactory efficiency using aniline blue. Nevertheless, in preliminary tests, we were not able to find a stain that colored calanoids, cyclopoids and cladocerans with similar efficiency. Dressel et al. (1972) also report variable results in staining various planktonic crustacean taxa.

Our results showed a clear decrease of calanoid mortality rates with increasing oxygen concentration but no relation between cyclopoid mortality rates and oxygen concentration. In our quantitative analyses, no distinction between taxa was made within the orders of copepods. However, since 2009, *E. affinis* on average accounts for 85 % of calanoid abundance in the upstream freshwater zone of the Scheldt, so the obtained results validate the hypothesis that cyclopoids have a higher tolerance for low oxygen concentrations than the calanoid *E. affinis.* Cladocerans, on the contrary, show a similar dependence of their mortality rate on oxygen concentration as

calanoids, and had statistically the same average mortality rate. So for this order, the hypothesis was rejected.

As explained in the introduction, most studies on low oxygen concentration tolerance are carried out in the context of oxygen minimum layer occurrence, either in coastal or estuarine systems. Stalder and Marcus (1997) compared hypoxia tolerance for 3 calanoids (*Labidocera aestiva, Acartia tonsa* and *Centropages hamatus)* under laboratory conditions, but few studies have specified differences in low oxygen concentration tolerance between different orders of zooplankters. In their Gulf of Mexico study, the groups of zooplankters found by Elliot et al. (2012) as occurring under more or less hypoxic conditions include either both calanoids and cladocerans or calanoids and cyclopoids.

Keister and Tuttle (2013) showed that in Hood canal, a sub-basin of Puget sound (USA), the calanoid *Paracalanus parvus* was generally found above the oxycline, while another calanoid, *Metrida pussilus* occurred below the oxycline. Their observation that the vertical positioning of the cyclopoid *Oithona similis* showed no relation to the oxycline is in line with our findings of independence of mortality rates of cyclopoids to oxygen concentration, but it should be mentioned that the only cyclopoid species present in the Scheldt water used for the experiments was *Acanthocyclops trajani*.

In the eutrophic Naka-umi lake (Japan) lake, Chang et al. (2013) studied zooplankton abundance and composition in an area where oxygen-rich water was infiltrated in the bottom to reduce eutrophication. The mesozooplankton dominating calanoid during spring, *Acartia hudsonica*, showed a ten-fold increase in abundance at the oxygenated site compared to a control, non-oxygenated site. During summer, no significant effect of oxygen addition was observed on the abundance of the dominant mesozooplankter, the cyclopoid *Oithona* spp. These results also agree with our results showing cyclopoids to be less sensitive to oxygen concentration than calanoids. In fact, *Oithona* spp. have been suggested as indicators of eutrophication and low oxygen concentration by several authors (Paffenhöfer, 1993; Roman and al., 1993, Richard and Jamet, 2001). Keister and Tutle (2013) explain the tolerance of *Oithona* spp. for low oxygen concentrations by its food preference for flagellates, which can dominate over diatoms in eutrophied areas (Uye, 1994) and the fact that *Oihona* carries its eggs and can thus avoid their sedimentation to hypoxic layers.

Our results showing a higher hypoxia tolerance for cyclopoids than for calanoids explains why the former were abundant in the upstream Scheldt prior to water quality improvement, but they do not explain the strong decrease in cyclopoid abundance since 2007. A detailed multifactor analysis of the zooplankton community changes in the upstream Scheldt and their possible causes is discussed in Chambord et al. (accepted).

It is also surprising that cladocerans which are known as tolerant for low oxygen concentration, in our experiments display a similar mortality rate as calanoids. The dominant cladoceran taxa in the experiments were *Bosmina longirostris* and *Daphnia galeata*. *Daphnia obtusa, Chydorus sphaericus* and *Ceriodaphnia quadrangula* were also present, but less abundant.

B. longirostris is known as typical for eutrophic, so oxygen poor systems and also to be more tolerant than other cladocerans to a large number of stresses including changing environmental conditions (Adamczuk, 2016). However, Bosminidae are suspected to include cryptic species (Taylor, 2002) and it is possible the *B. longirostris* having developed in the oxygenated freshwater Scheldt are a subspecies which has adapted to high oxygen concentration and lost its tolerate for lower oxygen concentration. Heisey and Porter (1977) showed that *Daphnia galeata,* the other dominant cladoceran in our experiments is less tolerant to environments with low oxygen concentration than *Daphnia magna*. This low tolerance of *D. galeata* is possibly due to a lower hemoglobin content.

Several authors suggest that hypoxia avoidance and/or differences in tolerance for hypoxia could have important implications for zooplankton community composition, positioning in space and trophic functioning (e.g. Sedlacek et Marcus, 2005; Richmond et al., 2006; Criales-Hernadez et al., 2008; Elliot et al., 2012). However, the development of clear scenarios on this topic is hampered by an important variability of oxygen related behavior and tolerance among zooplankton taxa and among development stages (Keister et Tutle, 2013).

Our results on low oxygen concentration tolerance of three different orders of zooplankters, combined with the evolution of the zooplankton community in the Scheldt estuary following water quality improvement and associated oxygen concentration increase, clearly show that hypoxia tolerance is an important factor in

determining zooplankton community composition in the freshwater Scheldt estuary. The development of *E. affinis* in this area was only possible since oxygen concentrations in the upstream area permitted it. Once *E. affinis* could develop upstream, it rapidly became a dominant species, as observed in several other systems (Counahan et al. 2005; Vad et al., 2012). Davidson et al. (2000), studying the zooplankton communities in various floodplain habitats of the Atchfalaya river (USA), also observe strong dominance of a few zooplankton taxa in well oxygenated sites, compared to more detritus rich sites, where zooplankton abundance was lower, but more diverse and evenly representing various taxa. So it can be envisaged that the dominance of *E. affinis* in the upstream Scheldt is perhaps not typical for this species, but a more general pattern of community diversity in relation to environmental conditions.

III.2.6 References

A, B --

Adamczuk, M. (2016). Past, present, and future roles of small cladoceran Bosmina longirostris (OF Müller, 1785) in aquatic ecosystems. Hydrobiologia, 767(1), 1-11.

Appeltans, W., Hannouti, A., van Damme, S., Soetaert, K., Vanthomme, R. & Tackx, M. (2003). Zooplankton in the Schelde estuary (Belgium/The Netherlands). The distribution of *Eurytemora affinis*: effect of oxygen? Journal of Plankton Research, 25(11), 1441-1445.

Baeyens, W., Van Eck, B., Lambert, C., Wollast, R. & Goeyens, L. (1998). General description of the Scheldt estuary. In Trace Metals in the Westerschelde Estuary: A Case-Study of a Polluted, Partially Anoxic Estuary (pp. 1-14). Springer Netherlands.

Bickel, S. L., Tang, K. W. & Grossart, H. P. (2009). Use of aniline blue to distinguish live and dead crustacean zooplankton composition in freshwaters. Freshwater Biology, 54(5), 971-981.

Bonvillain, C. P., Rutherford, D. A., & Kelso, W. E. (2015). Effects of environmental hypoxia on population characteristics of red swamp crayfish *Procambarus clarkii* in the Atchafalaya River Basin, Louisiana. Hydrobiologia, 743(1), 309-319.

C --

Chang, K. H., Imai, H., Ayukawa, K., Sugahara, S., Nakano, S. I. and Seike, Y. (2013). Impact of improved bottom hypoxia on zooplankton community in shallow eutrophic lake. Knowledge and Management of Aquatic Ecosystems, (408), 03.

Counahan, M. L., Andrews, R. M. & Speare, R. (2005). Reliability of parental reports of head lice in their children. Medical Journal of Australia, 182(3), 137.

Cox, T. J. S., Maris, T., Soetaert, K., Conley, D. J., Van Damme, S., Meire, P. & Struyf, E. (2009). A macro-tidal freshwater ecosystem recovering from hypereutrophication: the Schelde case study. Biogeosciences, 6, 2935-2948.

Criales-Hernández, M. I., Schwamborn, R., Graco, M., Ayón, P., Hirche, H. J. & Wolff, M. (2008). Zooplankton vertical distribution and migration off Central Peru in relation to the oxygen minimum layer. Helgoland Marine Research, 62(1), 85-100.

D, E --

Davidson Jr, N. L., Kelso, W. E. & Rutherford, D. A. (2000). Characteristics of cladoceran and copepod communities in floodplain habitats of the Atchafalaya River Basin. Hydrobiologia, 435(1-3), 99-107.

Dressel, D. M., Heinle, D. R. & Grote, M. C. (1972). Vital staining to sort dead and live copepods1, 2, 3. Chesapeake Science, 13(2), 156-159.

Elliott, D. T., Pierson, J. J. & Roman, M. R. (2012). Relationship between environmental conditions and zooplankton community structure during

summer hypoxia in the northern Gulf of Mexico. Journal of plankton research, 34(7), 602-613.

Elliott, D. T., Pierson, J. J. & Roman, M. R. (2013). Predicting the effects of coastal hypoxia on vital rates of the planktonic copepod *Acartia tonsa* Dana. PloS one, 8(5), e63987.

F --

Fox, H. M. (1948). The haemoglobin of Daphnia. Proceedings of the Royal Society of London B: Biological Sciences, 135(879), 195-212.

Fox, H. M., Gilchrist, B. M. & Phear, E. A. (1951). Functions of haemoglobin in Daphnia. Proceedings of the Royal Society of London B: Biological Sciences, 138(893), 514-528.

Fox, H. M. (1955). The effect of oxygen on the concentration of haem in invertebrates. Proceedings of the Royal Society of London B: Biological Sciences, 143(911), 203-214.

Fox, H. M. (1957). Haemoglobin in the Crustacea. Nature (Lond.) 179, 148.

G, H --

Green, J. (1959). Hæmoglobin and the habitat of the harpacticoid copepod *Elaphoidella gracilis* (Sars). Nature (Engl.) 183, 1834.

Heip, C. (1988). Biota and abiotic environment in the Westerschelde estuary. Hydrobiological Bulletin, 22(1), 31-34.

Heisey, D. & Porter, K. G. (1977). The effect of ambient oxygen concentration on filtering and respiration rates of *Daphnia galeata* mendotae and *Daphnia magna*. Limnology and Oceanography, 22(5), 839-845.

Hourdez, S., Lamontagne, J., Peterson, P., Weber, R. E. & Fisher, C. R. (2000). Hemoglobin from a deep-sea hydrothermal-vent copepod. The Biological Bulletin, 199(2), 95-99.

K, M --

Keister, J. E. & Tuttle, L. B. (2013). Effects of bottom-layer hypoxia on spatial distributions and community structure of mesozooplankton in a sub-estuary of Puget Sound, Washington, USA. Limnol. Oceanogr, 58(2), 667-680.

Kimmel, D. G., Miller, W. D., Harding Jr, L. W., Houde, E. D. & Roman, M. R. (2009). Estuarine ecosystem response captured using a synoptic climatology. Estuaries and Coasts, 32(3), 403-409.

Marcus, N. H., Richmond, C., Sedlacek, C., Miller, G. A. & Oppert, C. (2004). Impact of hypoxia on the survival, egg production and population dynamics of Acartia tonsa Dana. Journal of Experimental Marine Biology and Ecology, 301(2), 111-128.

Marcus, N. H. (2005). Calanoid copepods, resting eggs, and aquaculture. In: Copepods in aquaculture (ed. by C.-S. Lee, P.J. O'Bryen, N.H. Marcus), pp 3-9. Blackwell Publishing, Iowa USA.

Meire, P., Ysebaert, T., Van Damme, S., Van den Bergh, E., Maris, T. & Struyf, E. (2005). The Scheldt estuary: a description of a changing ecosystem. Hydrobiologia, 540(1-3), 1-11.

Mialet, B., Azémar, F., Maris, T., Sossou, C., Ruiz, P., Lionard, M., Van Damme, S., Lecerf, A., Muylaert, K., Toumi, N. & Tackx, M. (2010). Spatial spring distribution of the copepod *Eurytemora affinis* (Copepoda, Calanoida) in a restoring estuary, the Scheldt (Belgium), Estuarine, Coastal and Shelf Science, 88, 116-124.

Mialet, B., Gouzou, J., Azémar, F., Maris, T., Sossou, C., Toumi, N. & Tackx, M. (2011). Response of zooplankton to improving water quality in the Scheldt estuary (Belgium). Estuarine, Coastal and Shelf Science, 93(1), 47-57.

Moyson, S., Liew, H. J., Diricx, M., Sinha, A. K., Blust, R. & De Boeck, G. (2015). The combined effect of hypoxia and nutritional status on metabolic and ionoregulatory responses of common carp (*Cyprinus carpio*). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 179, 133-143.

P, R --

Paffenhöfer, G. A. (1993). On the ecology of marine cyclopoid copepods (Crustacea, Copepoda). Journal of Plankton Research, 15(1), 37-55.

Pirow, R. & Buchen, I. (2004). The dichotomous oxyregulatory behaviour of the planktonic crustacean *Daphnia magna*. The Journal of experimental biology, 207(4), 683-696.

Richard, S. & Jamet, J. L. (2001). An unusual distribution of *Oithona nana* Giesbrecht (1892) (Crustacea: Cyclopoida) in a bay: the case of Toulon Bay (France, Mediterranean Sea). Journal of Coastal Research, 957-963.

Richmond, C., Marcus, N. H., Sedlacek, C., Miller, G. A. & Oppert, C. (2006). Hypoxia and seasonal temperature: Short-term effects and long-term implications for *Acartia tonsa* Dana. Journal of Experimental Marine Biology and Ecology, 328(2), 177-196.

Roman, M. R., Gauzens, A. L., Rhinehart, W. K. & White, J. R. (1993). Effects of low oxygen waters on Chesapeake Bay zooplankton. Limnology and Oceanography, 38(8), 1603-1614.

S --

Sautour, B. & Castel, J. (1995). Comparative spring distribution of zooplankton in three macrotidal European estuaries. Hydrobiologia, 311(1-3), 139-151.

Sedlacek, C. & Marcus, N. H. (2005). Egg production of the copepod *Acartia tonsa*: The influence of hypoxia and food concentration. Journal of experimental marine biology and ecology, 318(2), 183-190.

Sell, A. F. (2000). Life in the extreme environment at a hydrothermal vent: haemoglobin in a deep-sea copepod. Proceedings of the Royal Society of London B: Biological Sciences, 267(1459), 2323-2326.

Soetaert, K. & Van Rijswijk, P. (1993). Spatial and temporal patterns of the zooplankton in the Westerschelde estuary. Marine Ecology Progress Series. 97(1), 47-59.

Stalder, L. C. & Marcus, N. H. (1997). Zooplankton responses to hypoxia: behavioral patterns and survival of three species of calanoid copepods. Marine Biology, 127(4), 599-607.

Svetlichny, L. & Hubareva, E. (2002). Effect of oxygen concentration on metabolism and locomotory activity of Moina micrura (Cladocera) cultured under hypo- and normoxia. Marine Biology, 141(1), 145-151.

T, U --

Tackx, M. L., De Pauw, N., Van Mieghem, R., Azémar, F., Hannouti, A., Van Damme, S. & Meire, P. (2004). Zooplankton in the Schelde estuary, Belgium and The Netherlands. Spatial and temporal patterns. Journal of Plankton Research, 26(2), 133-141.

Tang, K. W., Freund, C. S. & Schweitzer, C. L. (2006). Occurrence of copepod carcasses in the lower Chesapeake Bay and their decomposition by ambient microbes. Estuarine, Coastal and Shelf Science, 68(3), 499-508.

Terwilliger, N. B. & Ryan, M. (2001). Ontogeny of crustacean respiratory proteins. American zoologist, 41(5), 1057-1067.

Uye, S. I. (1994). Replacement of large copepods by small ones with eutrophication of embayments: cause and consequence. Hydrobiologia, 292(1), 513-519.

V, W --

Vad, C. F., Horvath, Z., Kiss, K. T. & Acs, E. (2012). Microcrustacean (Cladocera, Copepoda) communities in artificial lakes in the region of the North Hungarian Mountains, with special reference to the adventive species. Acta Zoologica Academiae Scientiarum Hungaricae, 58(1), 47-61.

Van Damme, S., Struyf, E., Maris, T., Ysebaert, T., Dehairs, F., Tackx, M. & Meire, P. (2005). Spatial and temporal patterns of water quality along the estuarine salinity gradient of the Scheldt estuary (Belgium and The Netherlands): results of an integrated monitoring approach. Hydrobiologia, 540(1-3), 29-45.

Wu, R. S. S., Lam, P. K. S. & Wan, K. L. (2002). Tolerance to, and avoidance of, hypoxia by the penaeid shrimp (Metapenaeus ensis). Environmental Pollution, 118(3), 351-355.

IV. CHAPITRE 4 :

Sélectivité trophique du copépode calanoïde *Eurytemora affinis* en eau douce dans l'estuaire de l'Escaut

IV.1 Résumé

IV.1.1 Contexte et objectifs

L'amélioration de la qualité de l'eau dans l'estuaire de l'Escaut, et plus particulièrement dans le tronçon d'eau douce, s'est manifestée à travers certains changements des facteurs physico-chimiques, tels que l'augmentation des concentrations en oxygène parallèlement à la diminution des concentrations en NH_4^+. La production primaire ayant augmenté, la silice est récemment devenue limitante pour la production des diatomées.

Le suivi de l'abondance et de la composition des communautés de zooplancton depuis 1996, dans le cadre du projet OMES, montrait à l'origine une communauté constituée principalement en eau saumâtre par les copépodes calanoïdes (dominée par *E. affinis*), et par les rotifères et les cladocères en amont, dans le tronçon d'eau douce.

Un changement important est survenu en 2007, avec le développement d'*E. affinis* en eau douce. Depuis, *E. affinis* est présent en abondance élevée dans ce tronçon, et est devenu largement dominant en termes d'abondance au sein de la communauté de copépodes, tandis que les copépodes cyclopoides ont fortement diminué.

Malgré la faible contribution du phytoplancton vivant à la matière en suspension estuarienne, l'importance de l'alimentation herbivore du zooplancton estuarien a été démontrée a plusieurs reprises (Gasparini et al., 1999 ; Tackx et al., 2003). Considérant la diminution des concentrations en silice dans la partie amont de l'estuaire de l'Escaut, la question de la capacité de la communauté de zooplancton dominée par *E. affinis* à contrôler de potentiels blooms phytoplanctoniques non-diatomiques se pose.

Cette étude a pour objectif de quantifier la pression de prédation du zooplancton sur le phytoplancton. A cet effet, des expériences d'incubation ont été réalisées. De l'eau naturelle de l'Escaut filtrée à 250 µm a été incubée avec et sans ajout d'*E. affinis* adultes et CV. Les pigments algaux ont ensuite été quantifiés par HPLC afin de déterminer l'impact des copépodes sur le phytoplancton.

Les résultats de ces expériences d'incubation ont été comparés à ceux obtenus par quantification des pigments intestinaux d'*E. affinis*.

IV.1.2 Principaux

Les expériences d'incubation suivies de la quantification pigmentaire par HPLC ont révélé une sélection des diatomées par *E. affinis*. A l'inverse, une augmentation de la quantité d'algues vertes a été observée, une croissance semblant stimulée par la présence d'*E. affinis* dans les flacons de broutage.

La méthode des contenus stomacaux a livré des résultats différents, avec une sélection des cryptophytes. Cependant, les deux méthodes ont abouti à des résultats comparables pour ce qui est des taux d'ingestion de chlorophylle *a* par *E. affinis*.

Nos résultats montrent un impact limité de la population d'*E. affinis* sur le stock phytoplanctonique et les diatomées dans le tronçon d'eau douce de l'Escaut estuarien, impliquant que la nourriture n'est pas un facteur limitant pour E. affinis. Par conséquent le zooplancton n'est probablement pas limitant pour le développement des niveaux trophiques supérieurs dans la partie d'eau douce de l'estuaire.

Compte tenu des différents résultats obtenus par les expériences d'incubation et la méthode des contenus stomacaux, l'impact d'*E. affinis* sur le phytoplancton non-diatomique est moins évident.

Les expériences d'incubation de microzooplancton (50-250µm) ont montré un impact surtout sur les phéopigments, suggérant une consommation importante des détritus. L'impact du microzooplancton sur le phytoplancton vivant n'a pas démontré de tendance régulière de sélectivité, soulignant la nécessité d'études supplémentaires à ce sujet.

IV.2 Article 3: Feeding selectivity of the calanoid copepod *Eurytemora affinis* in freshwater of the Scheldt

Feeding selectivity of the calanoid copepod *Eurytemora affinis* in freshwater of the Scheldt estuary

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IV.2.1 Abstract

Water quality in the Scheldt estuary has substantially improved since two decades, especially in the upstream, freshwater zone. The main effects in this regard are the improving oxygen concentration and in parallel, a reduction of NH_4^+ concentration. Primary production has increased, and recently, silica is becoming occasionally limiting for diatom production.

Zooplankton abundance and community composition has been monitored since 1996 in the frame of the OMES project, between Gent and the Belgian-Dutch boarder. The original spatial zooplankton distribution, showed mainly calanoid copepods (dominated by *E. affinis*) in the brackish water stretch and rotifers, cyclopoid copepods and cladocerans in the upstream, freshwater stretch. In 2007, an abrupt change was observed, as *E. affinis* developed massively in the freshwater area. Since then, *E. affinis* has maintained high abundance upstream and has become strongly dominant within the copepod community. Cyclopoids, on the other hand, have very strongly decreased in abundance.

Despite the small contribution of live phytoplankton to estuarine SPM, several studies indicate a substantial herbivorous feeding mode of estuarine zooplankton (Gasparini et al., 1999; Tackx et al., 2003). In view of the decreasing silica concentrations in the upstream Scheldt, the question arises in how far *E. affinis* as the dominate species of the zooplankton community will be capable of controlling potential non-diatom phytoplankton blooms.

This study therefor focused on the quantification of zooplankton feeding on phytoplankton. Incubation experiments using natural Scheldt water < 250 µm with and without addition of adult and CV *E. affinis* in natural Scheldt water were realized. HPLC quantification of algal pigments showed that *E. affinis* selected mainly diatoms. Green algae, on the other hand, showed growth –stimulated by the presence of *E. affinis* in the grazing bottles. This method was compared with the results of HPLC gut content quantification of *E. affinis*, which revealed, on the contrary to the incubation results, a selectivity for cryptomonads. *E. affinis* ingestion rates on Chl *a*-(eq) obtained by both methods were comparable. The *E. affinis* population has a limited impact on the standing stock of phytoplankton and of the dominating diatoms in the freshwater Scheldt. This means that the *E. affinis* population is not food limited, and that the zooplankton is probably not limiting the potential for higher trophic level

development in the freshwater Scheldt. Considering the different results obtained on grazing on non-diatom phytoplankton taxa with incubation and gut pigment content method, the *E. affinis* impact on non-diatom phytoplankton is less clear. Also the grazing activity of the microzooplankton community (t_c<50µm and $t_{microzoo}$ <250 µm) was quantified by 4 incubation experiments using natural Scheldt water. The impact of the microzooplancton community was mainly on pheaopigments, suggesting an important consumption of detritus. Microzooplankton grazing on live phytoplankton taxa showed no regular selectivity pattern, but more experiments are needed on this topic.

Key words:

Trophic selectivity, zooplankton, phytoplankton, *E. affinis*, Scheldt estuary.

IV.2.2 Introduction

Estuaries are perceived as highly productive ecosystems because they are often nutrient rich and have multiple sources of organic carbon, including riverine and waste inputs and autochthonous primary production by vascular plants, macroalgae, phytoplankton, and benthic microalgae to sustain populations of heterotrophs.

The special status of estuaries with high concentrations of suspended matter can interfere with the feeding of pelagic organisms (Hart, 1988; Kirk and Gilbert, 1990; Jack et al., 1993; Miquelis, 1996). Indeed, in estuaries, zooplankton faces an important constraint to ensure its nutritional needs. The pool of potential food sources consisting mainly of detrital POM of low nutritional quality (Heinle et al., 1977) can hinder the selectivity of the zooplankton for potential preys (i.e. high quality food), such as phytoplankton and microzooplankton (Gasparini and Castel, 1999). In the freshwater part of the Scheldt mesozooplankton is since 2007 dominated by the calanoid copepod: *Eurytemora affinis* (Mialet et al., 2011; Chambord et al., accepted). *E. affinis* is known as being generally herbivorous (Gasparini and Castel, 1997) or opportunistically omnivorous (Hoffman et al., 2008) and its food ingestion is highly efficient even in hyper turbid environments such as estuaries (DeMott, 1988; Tackx et al., 1995, 2003). Tackx et al. (2003), demonstrated than *E. affinis*, in the brackish zone of the Scheldt estuary, exerted a higher feeding pressure on phytoplankton than on the total particulate matter.

Selection between detritic organic material and phytoplankton preys has been studied in several laboratory experiments, where copepods often showed a preference for phytoplankton (DeMott, 1988, 1995; Paffenhofer & Van Sant, 1985). Some studies highlight that zooplanktonic copepods are able to detect and subsequently select their preys by chemoreception (Poulet et Ouellet, 1982; Hammer et al., 1983; Martel et al., 2006).

The ecosystem of the Scheldt estuary has been studied during the last years, especially the freshwater part. The freshwater tidal part extending over more than 80 km is one of the few remaining freshwater tidal habitats in Europe (Meire et al., 2005; Van Damme et al., 2005). The Scheldt estuary has historically been one of the most polluted in Europe (Heip, 1988; Meire et al., 2005). Since the 1980s, waste water treatment and regulation of pollutant discharge by industries led to an important improvement of the water quality (Van Damme et al., 2005; Cox et al., 2009). As a

consequence, oxygen concentrations increased while nutrient concentrations (i.e. NH_4^+, PO_4^-) decreased concomitantly. Since 2009, the morphology of the estuary has also changed due to its deepening between the mouth and Antwerp harbour, leading to an increase of the tidal pumping and increased salinity and SPM concentration in the upstream reaches (OMES, unpublished data).

In response to the improvement of water quality, the zooplankton community has experienced significant changes (Appeltans et al, 2003; Tackx et al., 2004; Mialet et al, 2010, 2011). Rotifers, and cladocerans and cyclopoid copepods represented the dominant taxa of the freshwater community (i.e upstream of Antwerp) between 1996 and 2006. Since 2007, a different zooplankton community has developed in the tidal freshwater reach with *E. affinis* becoming dominant and achieving higher densities than before in the brackish part. Concomitantly, cyclopoid copepods decreased to very low abundances. Only the abundance of cladocerans in fresh and brackish waters has remained constant. So, particular attention has been paid to the study of the zooplankton community (Mialet et al., 2011; Chambord et al., accepted). Furthermore, in the Scheldt estuary, the phytoplankton community has also changed. The biomass of diatoms and green algae has been increasing since 2006 and a change in the diatom community composition has been noted. Indeed, since 2009 the trend of the variation of the biomass of two taxa of diatoms has been reversed, i.e. *Actinocycluc normannii*, the most abundant species before 2009, decreased while the genus C*yclotella* spp. increased in abundance, inducing a change in size of diatoms (PAE-OMES data).

Predator-prey relationships between zooplankton and phytoplankton which in estuarine areas plays a key role in the transfer of energy between primary producer and higher trophic levels (Steele, 1974, Tackx et al., 2003; Maes et al., 2005b). They determine the potential contribution of primary production to higher trophic levels, and in estuaries, the efficiency of the uptake of primary produced material also influences the loss of primary production through export.

Climate change, and other impacts caused by humans can result in changes in the aquatic environments and thus in the configuration of food webs (e.g. Elliott & Quinto, 2007). Thus, a fundamental understanding of the mechanisms behind energy transfer through the food web is essential for sustainable management of biological resources. Diatoms were long time considered as not always dominant, but at least, an

important food source for copepods (e.g. Irigoien et al., 2000; Pasternak & Schnack-Schiel., 2001). However, the role of diatoms as the dominant food for copepods was challenged in the late 1990s when negative effects of certain diatom species on egg production and hatching success were found (Miralto et al., 1999; Turner et al., 2001). More recent findings contradict, however, these studies (Dutz et al., 2008; Jonasdottir et al., 2011). Selective feeding can have profound consequences for food fluxes (Kagata and Ohgushi 2011). For example, zooplankton selective grazing affects nutrient recycling through the removal of nutrient-rich species (Löder et al. 2011).

In the Scheldt estuary, the reduction of organic load enabled the upstream Scheldt estuary to change from a hypereutrophic system in which phytoplankton primary production was inhibited to a eutrophic system with substantial increase in primary production (Cox et al., 2009). During the last twenty years, substantial Chl *a* concentrations were observed, especially in the upstream reach (Fig. 25).

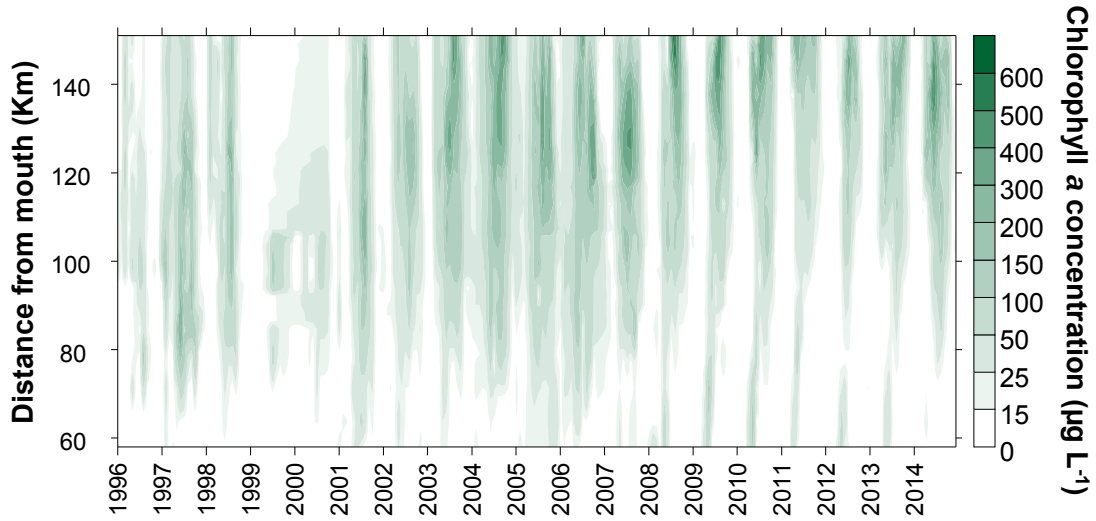


Figure 25 : Chl *a* concentrations in the Scheldt estuary, from station Melle (km 151) till Grens (km 51). OMES monitoring data, ECOBE, University of Antwerp.

During the same periods, low concentrations of dissolved silica (Dsi) became more frequent and were observed over of longer durations (Fig 26).

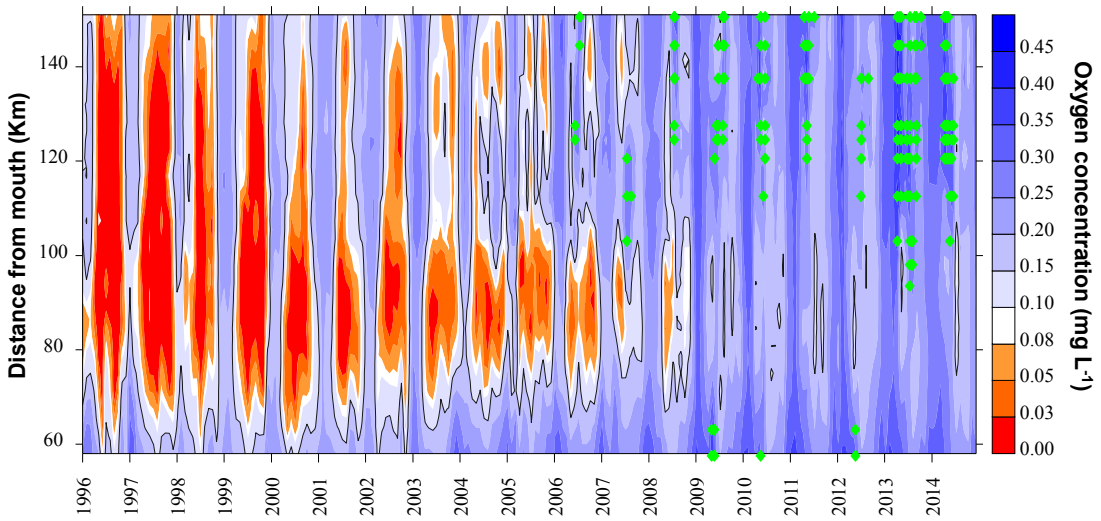


Figure 26 : *Oxygen concentrations in the Scheldt estuary, from station Melle (km 151) till Grens (km 51). The green stars indicate situations during which Dsi is potentially limiting diatom development. OMES monitoring data, ECOBE, University of Antwerp.*

Copepods are often reported to selectively feed on diatoms, and this is also the case for *E. affinis*, which has become dominant in the freshwater estuary since 2007. If Dsi limitation should limit diatom development, it is likely that non-diatom phytoplankton taxa, less consumable by a copepod dominated community, could become dominant. Also the recently increasing SPM concentration could enhance the development of non-diatom phytoplankton, as cryptomonads (Lionard et al., 2005).

The goal of the present study was to determine the impact of *E. affinis* on the present Scheldt phytoplankton community. Thus, in this paper we set out to answer the following questions:

1) What are the phytoplankton taxonomic groups which are selected by *E. affinis* as trophic sources, in the freshwater reach of the Scheldt estuary?
2) Considering decreasing silica concentrations in the upstream Scheldt, which could induce a decrease of diatom abundance, the question arises as how far the *E. affinis*- dominating the zooplankton community could be able to control potential non-diatom phytoplankton blooms.

The study of the diet of planktonic organisms in estuarine environments has been addressed using different methods: experiments by incubating predators and preys under controlled conditions or simulated *in situ* conditions (Gasparini and Castel, 1999; Winkler and Greve, 2004; David et al., 2006), trophic biomarker analyses such as pigments (Islam et al., 2005) fatty acids (Bodineau et al., 1998, Casvol et al., 2015)

and isotope ratios (Gladishef et al., 2016). All these techniques provide different approaches to trophic relationship study.

In the present study, we combined two complementary methods (i.e; experimental and field studies). First, grazing experiments using incubation of *E. affinis* in natural Scheldt water, combined with HPLC quantification of algal pigments in microcosm water incubated with and without *E. affinis*. Secondly, gut pigment content analyses on adult *E. affinis*. Copepods for gut pigment analyses and location.

IV.2.3 Material and methods

IV.2.3.1 Study site

The Scheldt has its source in the North of France and runs through Belgium to join the North Sea at Vlissingen in the Netherlands. Its estuary is situated from the mouth at Vlissingen until the city of Gent, where the tide is stopped by sluices (Fig. 27). Its four main tributaries are Boven Scheldt, Dender, Durme and Rupel. All the samples for this study were collected at a fixed station (Hemiksen, 51°14431 NS, 4°34605 OE) during two campaigns, one in April 2013 (9 experiments) and one in April 2014 (4 experiments) (Fig 27).

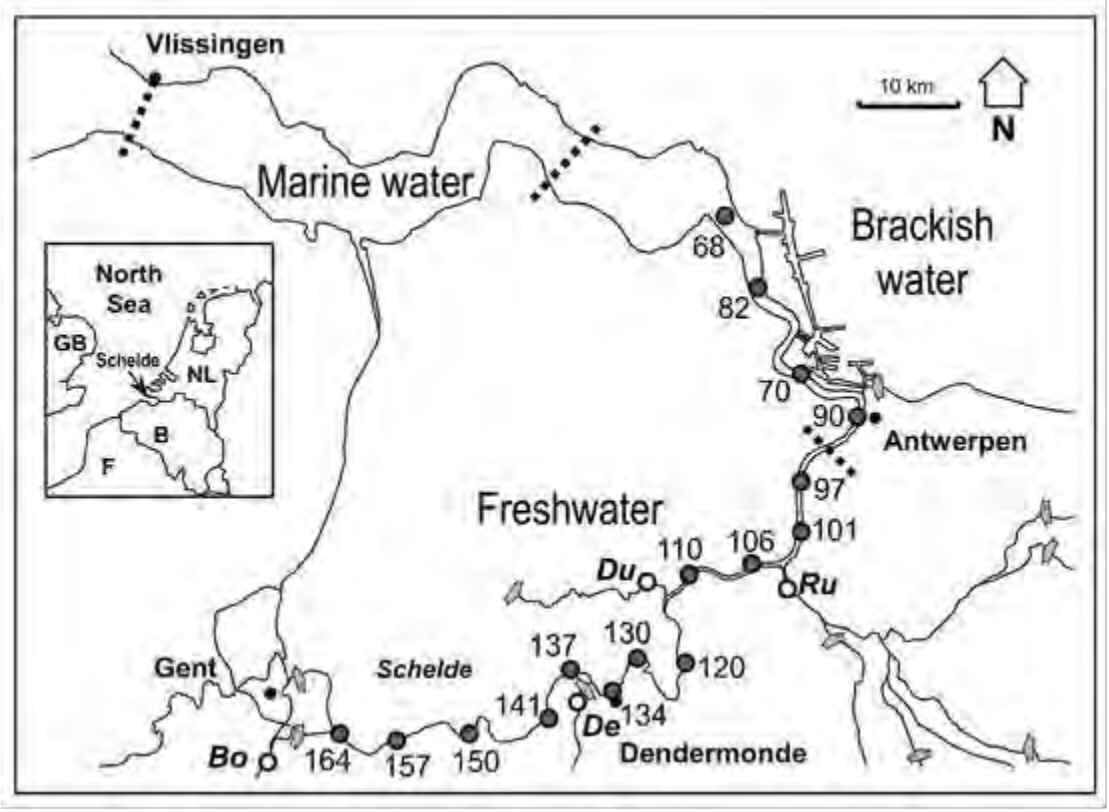


Figure 27 : Map of the Scheldt estuary with OMES sampling stations, designated by their distance, in km upstream from Vlissingen (mouth). Dotted lines indicate milits between marine water, brackish water and freshwater reaches. Sampling station Antwerpen indicated.

IV.2.3.2 Experimental procedure

IV.2.3.2.1 Field work

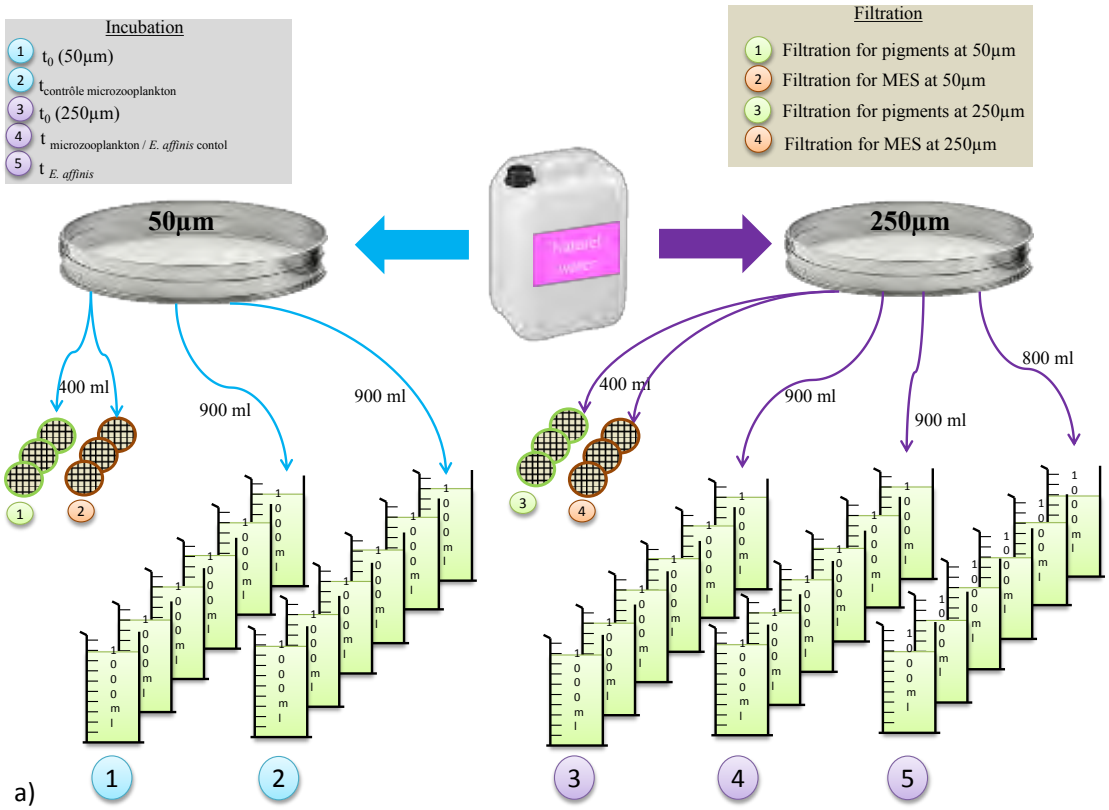
Conductivity, temperature and salinity of the natural water were measured at each sampling time using a Multi parameter sensor (WTW, Multi 3430). Water samples of 250- 450 ml, depending on the SPM load, were filtered on pre –weighed Whatman GF/C filters (DW filter) for the quantification of SPM and OM concentration in the water at the time of sampling.

For the incubation experiments, a concentrated zooplankton sample was collected at the beginning of each experiment by leaving a 150 µm mesh plankton net counter current at sub-surface for 15 minutes. The collected zooplankton was brought into a 1 liter container filled with of natural Scheldt water. At the same time, 50 L of natural water were also taken at surface and both zooplankton and water were transported to the laboratory.

For the gut pigment analysis, zooplankton was collected by filtering of sub-surface water with 150 µm mesh plankton net. The collected zooplankton was immediately frozen in liquid nitrogen. These frozen samples were stored at -80°C upon arrival in the laboratory until *E. affinis* sorting for gut pigment analyses.

IV.2.3.2.2 Incubation experiments

For logistic reasons, the both sets of incubation experiments (9 experiments in April 2013 and 4 in April 2014) (fig. 28a,b) were conducted in the ECOBE laboratory at Antwerp University.



a)

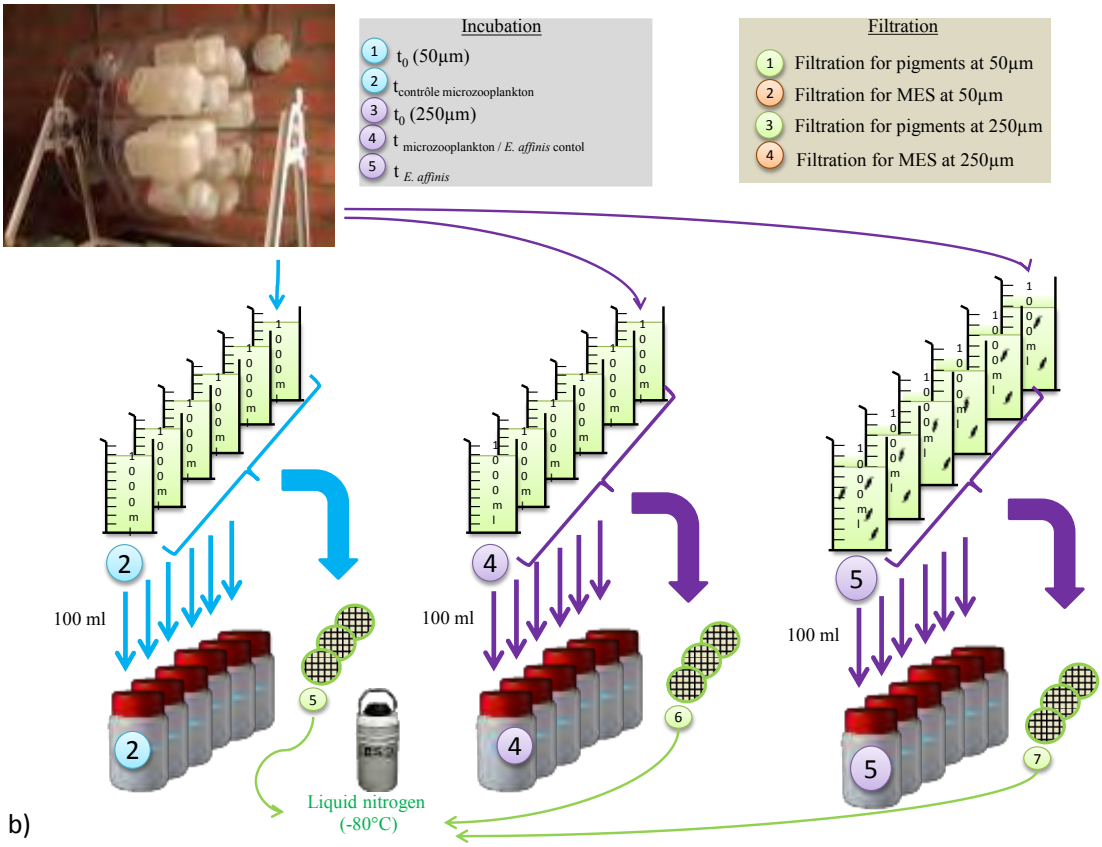


Figure 28: Experimental design for incubation experimentats. a) at the beginning of each experiment b) at the end of each experiment.

Living adults and CV (stage V for copepodites) individuals of *E. affinis* to be used in the experiments were carefully isolated from filtered water samples under stereomicroscope (90x magnification) using an ophthalmic surgical clamp and 6x 50 individuals and were brought into < 100 ml volumes of natural water filtered on 250 µm.

9 replicates per experiment were realized for 2013 experiments (18 for 2014 experiments). The natural water was filtered on 250 µm to remove zooplankton. Each microcosm (1 L polycarbonate bottle) was filled with 900 ml of this filtered water at time zero. For each experiment, there were 9 (18 for 2014) microcosms: 3 t_0 (6 for 2014) replicates and 3 (6 for 2014) control replicates (tc) containing only the filtered water, and, 3 (6 for 2014) treatment replicates (tz) 50 *E. affinis* per microcosms using 100 ml natural water filtered to adjust to the same 900 ml level as the control microcosms.

Once individuals introduced into the t_z microcosms, each bottle was closed and placed in a rotating incubator (2rpm) to prevent SPM sedimentation during the incubation (for 24 hours, in the dark). In 2013, the temperature was 11°C (as in the naturel environment). Due to technical problems, temperature was 19°C in 2014 whereas it averaged 11.7 °C in the field. At the beginning of the experiment (t0), 250 ml water samples were collected from the t_0 bottles and filtered on to Whatman GF/C glass fiber filters to measure the phytoplankton pigment concentration present in the Scheldt water at the start of the experiment. Filters were then stored in liquid nitrogen for further HPLC pigment analyses. At the end of the incubation, between 200 and 450 ml water samples collected from each bottle were filtered on GFC filters and these filters were also stored in liquid nitrogen prior to HPLC analysis. As in our experiments, the Scheldt water was filtered over 250 µm, it is likely that a substantial microzooplancton community was present in both control and grazing bottles. To verify for the intensity of microzooplancton feeding on phytoplankton, for each experiments microcosms filled with the water filtered over 50 µm, were also incubated: 3 t_0, tc and tz replicates. Sampling was carried out as described above and samples were stored in liquid nitrogen. Upon arrival in the EcoLab laboratory, all frozen samples were stocked in a -80 deepfreezer.

For SPM and OM quantification, filters were dried (45 °C), weighed for SPM and re-weighed to obtain Organic Matter (OM) concentrations.

IV.2.3.3 HPLC protocol

Extraction of individuals for the gut content as well as HPLC analysis were carried out at the Ecolab laboratory in Toulouse.

IV.2.3.3.1 Pigment extraction

For phytoplankton pigment extraction, each filter was extracted 2 times in a total volume 10 ml (5 and 5 ml) 98% cold-buffered methanol (with 2% of 1 M ammonium acetate) following Mialet et al. (2013) for 15 minutes at 20°C in darkness. Algal pigment release was favoured at each step by 60 seconds an ultrasonication

probe (Branson ultrasonic bath model 5810). Then, 1 ml of the total pigment solution was filtred (0.2 µm PTEE syringe filter) and HPLC analysed.

IV.2.3.4 Gut pigment measurements

E. affinis individuals (adults and CV) were isolated from the frozen samples using a needle picker, clamp and petri dish on ice and under minimum light exposure to prevent pigment degradation. Each individual was meticulously washed in a cold milliQ water bath to remove organic or sediment particles as much as possible and finally 30 individuals were placed in 10 µl of milliQ water. Following this procedure, when it was possible, 6 replicates were made (3 replicates of females and 3 replicates of males). Pigments were extracted from *E. affinis* samples by ultrasonication (Fisher scientific, FB15046), for 120 seconds in 300 µl of 98 % cold-buffered methanol (with 2 % ammonium acetate 1M) following Buffan-Dubau and Carman (2000a) and were then incubation for 15 minutes at 20°C in darkness. The total pigment solution was then filtered (0.2 µm PTEE syringe filter) and HPLC analysed.

IV.2.3.4.1 HPLC pigment quantification

HPLC analyses of *E. affinis* gut pigments and phytoplankton pigments were performed using a liquid chromatograph consisting of a 100 µL loop auto-sampler and a quaternary solvent delivery system coupled to a diode array spectrophotometer (LC1200 series, Agilent technologies inc., Santa Clara, CA, USA). The mobile phase was prepared and programmed according to the analytical gradient protocol described in Barlow et al. (1997). Pigment separation was performed through a C8, 5 µm column (MOS-2 HYPERSIL, Thermo Fisher scientific inc., Waltham, MA, USA). The diode array detector was set at 440 nm to detect carotenoids, at 665 nm for chlorophylls and pheopigments (Wright et al., 1991). Pigments were identified by comparing their retention time and absorption spectra with those of pure standard pigments (DHI LAB products, Hørsholm, Denmark). Each pigment concentration was calculated by relating the peak area of its chromatogram with the corresponding area of calibrated standard.

IV.2.3.5 Suspension and organic matter

Filters for SPM quantification were dried at 60 °C for 24 h, cooled in a desiccator and weiged (DWsample) on a Mettler balace (precision: mg). SPM (dry weight) concentration was calculated as the difference between DW sample and DW filter.

The same filters were afterwards burded at 500 °C in a Muffler oven, colled in a dessicator a reweighed for Ash weight (AW). The concentration of the organic matter (OM) in the samples was calculated as SPM-AW and expressed in mg L^{-1}

IV.2.3.6 Data analyses

Incubation experiments. Differences in mean pigment concentrations between control (t_c) and bottles with zooplankton (t_z) are considered significant when $p<0.05$ following Mann-Whitney tests.

For each pigment, clearance- and ingestion rates were calculated according to Frost (1972).

The grazing coefficient, g (h^{-1}) for each experimental bottle was calculated as:

$$g = \frac{1}{(t_2 - t_1)} \times ln\left(\frac{\overline{t_c}}{t_z}\right)$$

- t_1 is the time zero, t_2 the time end of the experiment (T°C)
- $\overline{t_c}$: mean pigment concentration (µg L^{-1}) at the end of the experiment in the control bottles,
- t_z: pigment concentration (µg L^{-1}) at the end of the experiment in each of the experimental bottles.

Clearance rate (F, ml $ind.^{-1}$ h^{-1}) was calculated from each experimental bottle as:

$$F = \frac{V \times g}{N}$$

where V is the volume (ml) of water of the experimental bottles and N is the number of copepods in the experimental bottles (t_z).

Ingestion rate (I, ng ind.$^{-1}$h^{-1}) of *E. affinis* on each pigment was calculated as:

$$\mathbf{I = C \times F}$$

with C, the concentration (µg L^{1}) in the experimental bottle, detailed below.

k: growth coefficient (h^{-1}):

$$k = \frac{1}{(t_2 - t_1)} \times ln\left(\frac{\overline{t_c}}{\overline{t_0}}\right) \quad (1)$$

$\overline{t_0}$: mean pigment concentration (µg L^{-1}) in the replicate t_0 bottles (2)

$$C = \frac{\overline{t_0} \times \left(e^{(k-g)(t_2 - t_1)} - 1\right)}{(t_2 - t_1)(k - g)} \quad (3)$$

Community ingestion rate (C µg ind.$^{-1}$h^{-1}) was calculated by multiplying individual ingestion rates by *E. affinis* adult and CV abundance in the field.

The clearance rate of the microzooplancton (t_0<50µm and $t_{microzoo}$ <250 µm), was also calculated following Frost, 1972, adapted as:

$$F = \frac{V_1}{t \times V_2} \times ln\left(\frac{\overline{t_c}}{t_z}\right) \quad ml\ L^{-1}h^{-1} \quad (4)$$

with : V_1 : incubation volume in microcosm (ml) V_2: 900 ml (4).

The use of Q_{10} has been necessary to correct the 2014 temperature difference between the incubation conditions and the field temperature at the time of sampling.

A Q_{10} of 2,5 (Durbin and Durbin, 1992; David et al., 2006) was therefore applied to the calculated clearance and ingestion rates as:

$$F_2 = F_1 \times Q_{10}^{\left(\frac{T_2 - T_1}{10}\right)}$$

- **F1** is the measured clearance or ingestion rate (I_1) at temperature T1 (where $T_1 > T_2$). T1 is the 2014 laboratory incubation temperature.
- **F2** is the measured clearance or ingestion rate (I_1) at temperature T2 (T2 is the natural water temperature.

For gut pigment content results, the ingestion rate (I) of *E. affinis* on phytoplankton was calculated by multiplying gut content (ng of pigment ind.$^{-1}$) by the Gut Clearance Rate (GCT), using the regression: GCT = 0.0117 + 0.001794 T (°C) (Dam and Peterson, 1988). Clearance rate (F) on each pigment was calculated by dividing the ingestion rate by the pigment concentration, measured in the water at the time of sampling.

IV.2.4 Results:

IV.2.4.1 Concentration and composition of the suspended matter

SPM concentration in the experiments varied from 18.03 ± 6.63 mgL^{-1} to 178.11 ± 52.10 mgL^{-1}, OM concentration between 5.65 ± 0.86 and 27.75 ± 31.49 mgL^{-1}. The SPM was composed for minimum 14.53 and maximum 31.87% of organic matter. Chl *a* concentration varied between 1.53 ± 0.09 to 3.83 ±0.51 µg L^{-1}. Estimating live phytoplankton contribution to OM as: Chl *a* x 30 x 2 (Lionard et al., 2008; OMES, PAE), the contribution of phytoplankton was between 0.4 and 2.11 % (fig. 29).

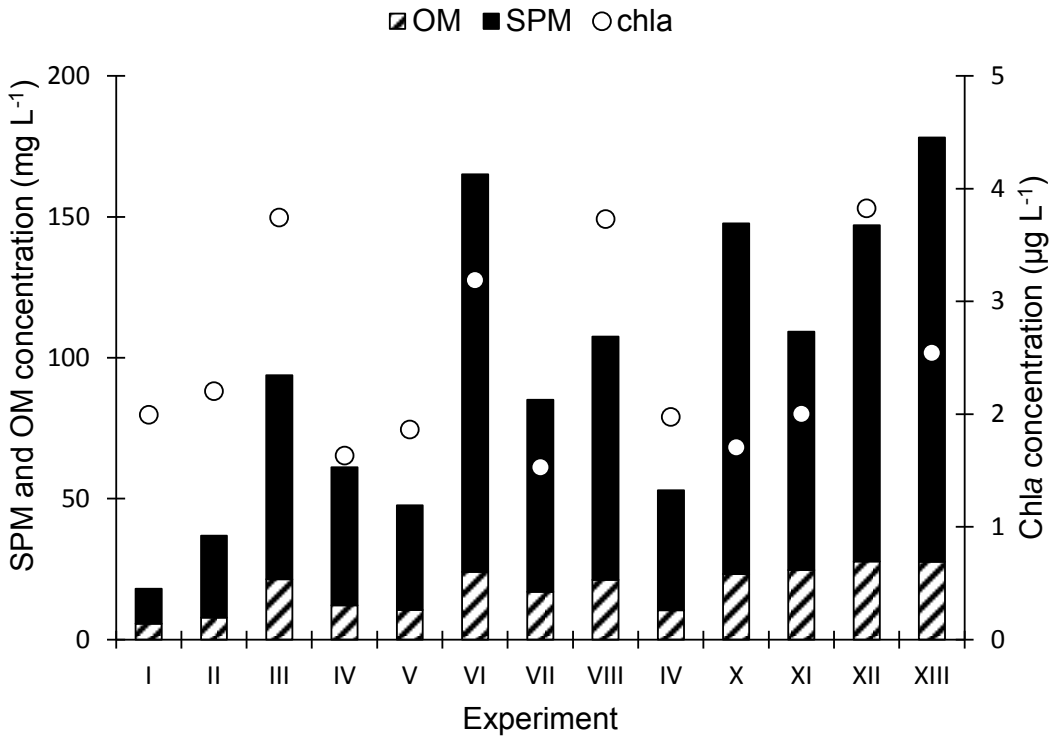


Figure 29 : Mean concentrations of SPM and OM in each experiment at t_0. Symbols: Chl *a* concentration

There was a significant correlation ($p<0,001$) between the SPM and OM concentrations in the natural filtered (on 250µm mesh) Scheldt water which was used for the incubation experiments at t_0 (Fig. 30a). However, there was no significant correlation between Chl *a* and OM concentrations at t_0 ($p>0,05$, Fig 30b).

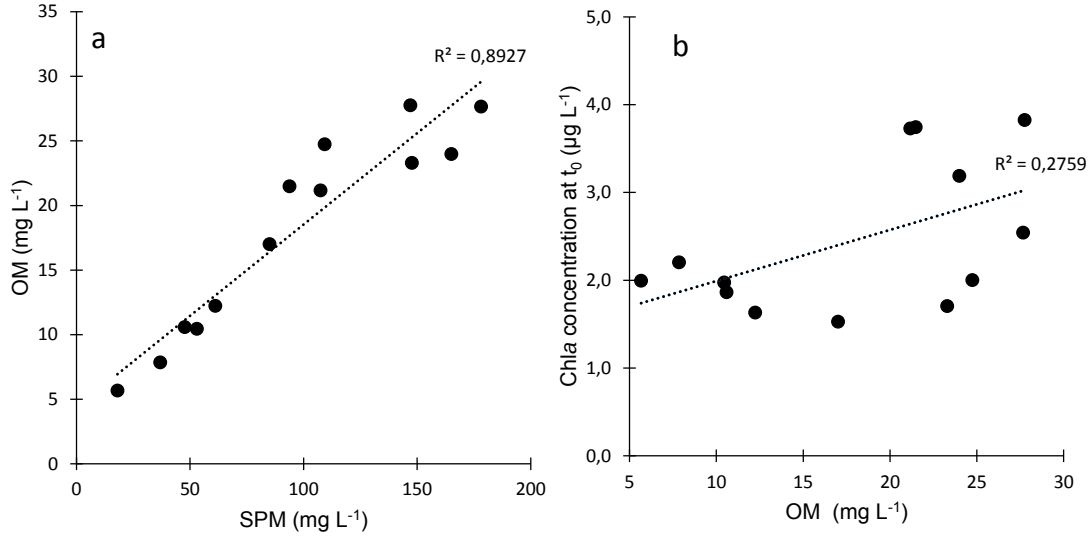


Figure 30 : Average concentrations of OM (n=3) as a function of average concentrations of SPM (n=3) (a) and average concentration Chl *a* as a function of OM concentrations (b) at t_0 in natural Scheldt water used for the incubation experiments.

IV.2.4.2 Pigment composition

The biomarker pigments which were detected in microcosm water were (A) chlorophyll *c*, fucoxanthin, diadinoxanthin and diatoxanthin, (B) alloxanthin and an unidentified pigment which is probably monadoxanthin, (C) lutein and violaxanthin, (D) zeaxanthin (Fig. 31a). These can be considered as biomarkers pigments for the following phytoplankton taxa: diatoms (A), cryptophytes (B), green algae (C), and cynobacteria/green algae (D) (Jeffrey et al., 1997).

Gut content analyses of *E. affinis* indicated that astaxanthin was the most concentrated pigment in all samples (Fig. 31b). This pigment is present in the body tissues of copepods (Matsuno, 1989). Cryptophytes (B) and diatoms (A) were represented by the biomarker pigments monadoxanthin and alloxanthin, and, diatoxanthin respectively. 26 % of the gut content analyses revealed the presence of diatoxanthin.

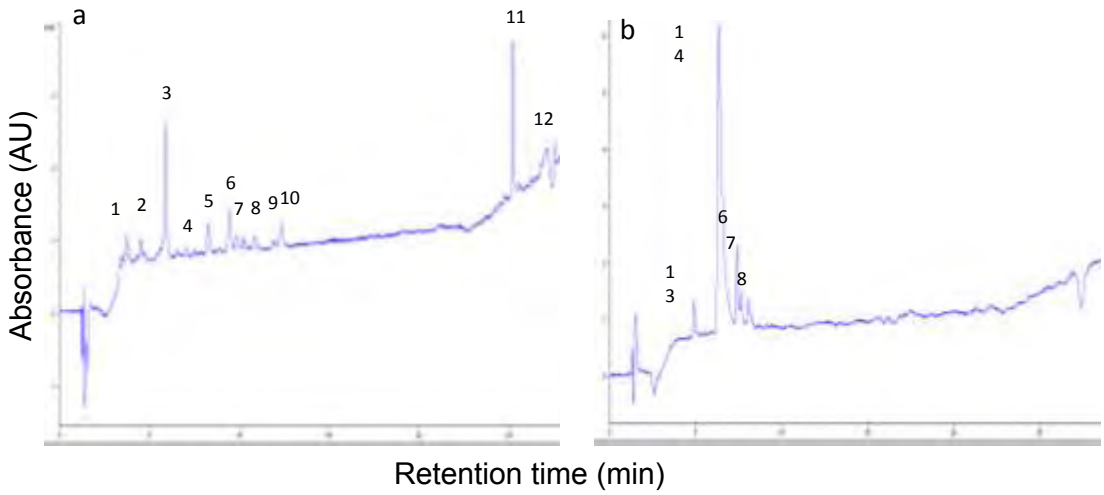


Figure 31 : Examples of HPLC absorbance chromatograms obtained at 440nm from samples. (a) incubation experiment, (b) gut content. 1: chlorophyll *c*; 2; pheophorbide *a*; 3: fucoxanthin; 4: violaxanthin; 5: diadinoxanthin; 6: possibly monadoxanthin; 7: alloxanthin; 8: diatoxanthin; 9: zeaxanthin; 10: lutein; 11: chlorophyll *a*; 12: β-carotene; 13: pheophorbide coeluted with unidentified pigment 14: asthaxanthin.

At t_0, in water samples, the more important biomarker pigment was fucoxanthin (mean concentration = 1.15 ±0.44 µg L^{-1}) (Fig. 32). Chl *a* concentration ranged from 1.53 ±0.09 to 3.83 ±0.51 µg L^{-1}.

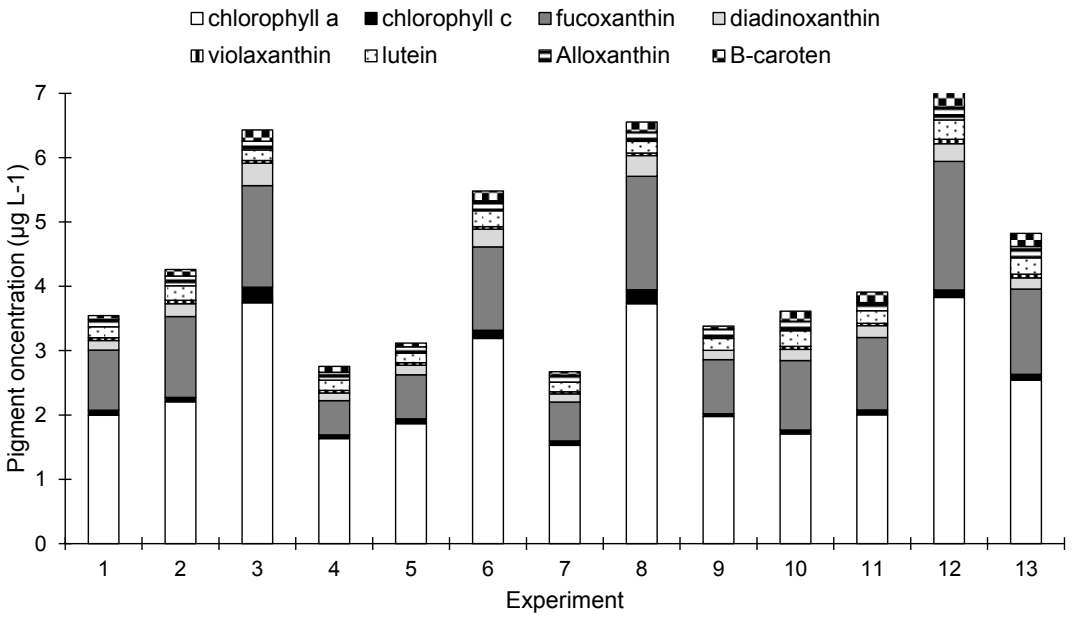


Figure 32 : Marker pigments mean concentration, in the natural Scheldt water used for incubation experiments at t_0 in all experiments (n=13).

Fucoxanthin concentrations were correlated to the concentrations of Chl *a* (p= 0.034), showing that diatom constituted the major constituent of the phytoplankton community (Fig. 33).

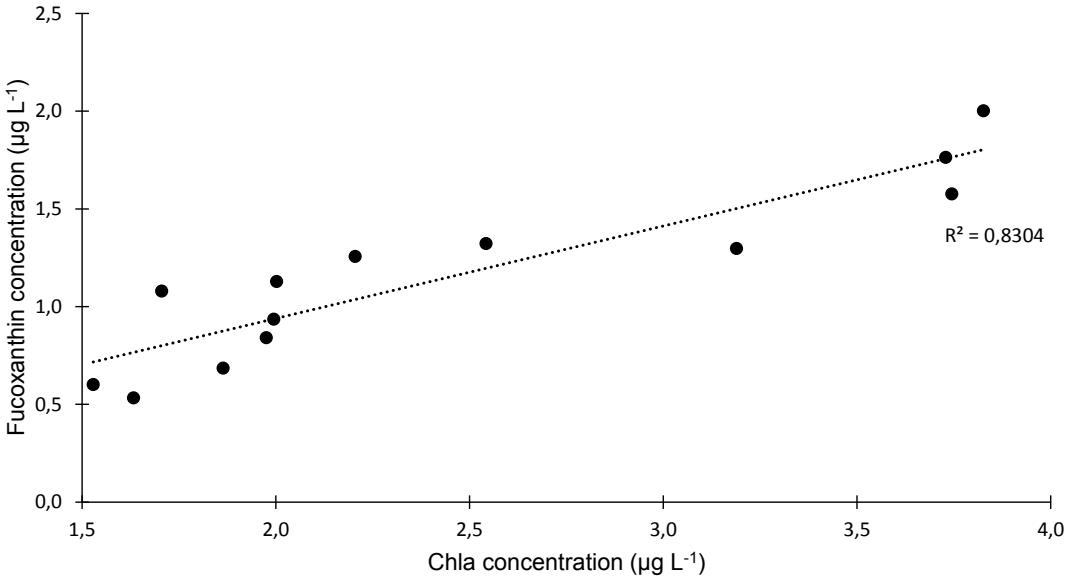


Figure 33 : Fucoxanthin concentration as a function of chlorophyll *a* concentration in natural Scheldt water at t_0.

Although, the diatom pigments, fuxoxanthin was not detected in gut contents, diatoxanthin was present (fig.30), indicating that diatoms were ingested by the copepod. Besides, the biomarker pigments originated from cryptophytes (peack: 8, 9,

Fig.31) were also found in gut contents (while green algal pigments (peack: 4, 9, 10, Fig.31) were not), indicating that the copepod also ingested cryptophytes, but likely no green algae.

IV.2.4.3 Clearance rates in incubation experiments with E. affinis

Over the 13 incubation experiments run with *E. affinis*, Chl *a* had a significant lower mean concentration ($p<0.05$) in the experimental bottles than in the control bottles in 61 % of the cases at the end of the experiment. This difference was also observed for the mean concentrations of the diatom pigments fucoxanthin, diadinoxanthin and Chl *c* in 54 % of the cases. In contrast, the mean concentrations of alloxanthin (a cryptophyte marker pigment) and lutein (a chlorophyte marker pigment) lutein were significantly higher in the experimental bottles than in the control bottles in 69 and 46 % of the experiments respectively. Other pigments showed less significant or repetitive differences in mean concentration between control and experimental bottles (Table 4).

Table 4 : Results of the 13 grazing experiments (from I to IX in 2013 and from X to XIII in 2014) with *E. affinis*. Mean concentrations of each pigments (n=3 for 2013; n=6 for 2014 ± standard error in annexe1) at t0, in

control (tcontrol) and treatment (tzoo) microcosms. Mean ingestion and clearance rates (n=3 for 2013; n=6 for 2014 ± standard deviation in annexe1)

Mean t_0 (µg L^{-1})												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
I	0.935	0.046	0.146	0.037	0.169	0.113	0.062	0.081	1.994	0.511	0.190	0.041
II	1.256	0.058	0.199	0.068	0.222	0.153	0.101	0.068	2.204	0.631	0.258	0.052
III	1.577	0.042	0.349	0.057	0.164	0.138	0.174	0.243	3.745	0.419	1.537	0.052
IV	0.532	0.040	0.117	0.083	0.162	0.120	0.093	0.059	1.633	0.318	1.958	0.037
V	0.685	0.039	0.150	0.065	0.148	0.097	0.059	0.075	1.864	0.255	1.539	0.045
VI	1.297	0.043	0.274	0.084	0.244	0.155	0.152	0.127	3.189	0.481	1.828	0.091
VII	0.601	0.034	0.127	0.069	0.150	0.107	0.053	0.069	1.529	0.264	1.226	0.141
VIII	1.763	0.042	0.320	0.052	0.181	0.142	0.159	0.219	3.729	0.375	1.162	0.073
IX	0.841		0.145	0.064	0.185	0.142	0.050	0.045	1.975	0.494	0.188	0.064
X	1.079	0.046	0.173	0.147	0.244	0.149	0.154	0.063	1.705	0.582	0.474	0.114
XI	1.129	0.037	0.182	0.112	0.196	0.126	0.163	0.075	2.002	0.461	0.285	0.061
XII	2.001	0.069	0.273	0.228	0.301	0.195	0.301	0.116	3.826	0.949	0.429	0.143
XIII	1.323	0.057	0.176	0.196	0.254	0.176	0.203	0.091	2.542	0.690	0.391	0.121

Mean tcontrol (µg L^{-1})												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
I	0.456	0.037	0.104	0.082	0.111	0.076	0.067	0.059	1.331	0.167	1.406	0.027
II	0.551	0.033	0.084	0.079	0.117	0.080	0.042	0.046	1.007	0.177	1.660	0.033
III	1.594	0.029	0.288	0.053	0.120	0.106	0.090	0.359	3.386	0.265	1.315	0.041
IV	0.602	0.039	0.112	0.076	0.146	0.100	0.077	0.061	1.524	0.272	1.875	0.046
V	0.748	0.048	0.130	0.061	0.145	0.098	0.060	0.067	1.761	0.218	1.397	0.044
VI	1.875	0.046	0.225	0.075	0.188	0.126	0.104	0.130	2.579	0.360	1.564	0.065
VII	0.692	0.051	0.118	0.060	0.189	0.106	0.075	0.082	1.929	0.307	1.448	0.063
VIII	1.956	0.044	0.331	0.059	0.183	0.125	0.094	0.235	3.611	0.319	1.209	0.069
IX	0.943		0.131	0.074	0.222	0.130	0.068	0.042	1.911	0.467	0.362	0.064
X	0.877	0.034	0.116	0.124	0.180	0.114	0.069	0.065	1.391	0.235	0.265	0.092
XI	1.063	0.032	0.141	0.101	0.187	0.098	0.125	0.074	1.891	0.440	0.253	0.061
XII	1.917	0.072	0.305	0.186	0.283	0.152	0.210	0.143	3.873	0.766	0.424	0.116
XIII	1.550	0.046	0.249	0.162	0.260	0.130	0.202	0.104	2.753	0.680	0.359	0.097

Mean tzoo (µg L^{-1})												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
I	0.428	0.099	0.096	0.070	0.116	0.083	0.081	0.080	1.400	0.227	1.553	0.056
II	0.472	0.033	0.076	0.091	0.115	0.088	0.054	0.033	0.978	0.195	1.783	0.034
III	0.885	0.037	0.214	0.056	0.166	0.150	0.194	0.109	2.410	0.475	2.183	0.053
IV	0.439	0.036	0.078	0.072	0.142	0.095	0.070	0.033	1.235	0.281	1.826	0.044
V	0.554	0.045	0.107	0.055	0.147	0.096	0.061	0.047	1.418	0.209	1.645	0.039
VI	0.846	0.040	0.161	0.075	0.199	0.150	0.108	0.074	1.879	2.588	1.785	0.072
VII	0.452	0.036	0.084	0.065	0.155	0.114	0.040	0.046	1.367	0.222	1.246	0.054
VIII	0.853	0.045	0.176	0.043	0.188	0.123	0.176	0.088	1.774	0.519	1.511	0.071
IX	0.709	0.059	0.094	0.072	0.199	0.146	0.078	0.031	1.538	0.463	0.285	0.064
X	0.871	0.038	0.120	0.147	0.217	0.154	0.128	0.039	1.503	0.510	0.369	0.092
XI	0.658	0.034	0.097	0.096	0.173	0.127	0.148	0.033	1.294	0.432	0.228	0.071
XII	0.972	0.046	0.128	0.156	0.225	0.141	0.219	0.049	2.002	0.763	0.407	0.106
XIII	1.033	0.037	0.150	0.154	0.240	0.166	0.194	0.056	1.850	0.713	0.397	0.105

Mean F (ml ind.$^{-1}$h^{-1})												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
I	0.065	-0.682	0.090	0.194	-0.017	-0.064	-0.136	-0.238	-0.021	-0.280	-0.066	-0.525
II	0.166	0.003	0.110	-0.135	0.031	-0.077	-0.123	0.358	0.052	-0.033	-0.055	0.026
III	0.582	-0.216	0.294	-0.047	-0.316	-0.341	-0.751	1.188	0.334	-0.533	-0.498	-0.251
IV	0.325	0.072	0.359	0.049	0.033	0.063	0.099	0.610	0.208	-0.025	0.029	0.063
V	0.301	0.062	0.193	0.106	-0.002	0.022	-0.003	0.377	0.216	0.046	-0.161	0.145
VI	0.783	0.154	0.329	-0.003	-0.050	-0.174	0.015	0.558	0.322	-0.873	-0.121	-0.087
VII	0.422	0.344	0.331	-0.052	0.199	-0.042	0.634	0.609	0.354	0.321	0.156	0.168
VIII	0.818	-0.023	0.628	0.318	-0.023	0.018	-0.617	0.979	0.701	-0.471	-0.218	-0.005
IX	0.280		0.333	0.027	0.106	-0.107	-0.120	0.297	0.214	0.008	0.241	0.011
X	0.016	-0.022	-0.001	-0.060	-0.071	-0.119	-0.258	0.231	-0.016	-0.324	-0.137	0.010
XI	0.217	-0.024	0.173	0.024	0.048	-0.112	-0.056	0.348	0.178	0.029	0.054	-0.051
XII	0.304	0.201	0.395	0.079	0.115	0.073	-0.004	0.464	0.294	0.008	0.024	0.039
XIII	0.195	0.093	0.249	0.030	0.050	-0.099	0.023	0.271	0.175	-0.003	-0.036	-0.009

Mean I (ng ind.$^{-1}$h^{-1})												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
I	0.041	-0.067	0.009	0.008	-0.006	-0.008	-0.014	-0.023	-0.080	-0.105	-0.070	-0.033
II	0.124	0.000	0.013	-0.011	0.004	-0.011	-0.018	0.016	0.048	-0.030	-0.059	-0.002
III	0.694	-0.008	0.081	-0.003	-0.053	-0.049	-0.138	0.195	1.012	-0.255	-0.918	-0.013
IV	0.151	0.003	0.034	0.004	0.005	0.006	0.007	0.027	0.294	-0.010	0.050	0.002
V	0.182	0.002	0.024	0.006	-0.002	0.002	-0.001	0.021	0.347	0.010	-0.256	0.005
VI	0.826	0.006	0.070	0.000	-0.012	-0.027	-0.005	0.055	0.774	-2.495	-0.242	-0.008
VII	0.220	0.012	0.034	-0.004	0.030	-0.008	0.028	0.033	0.488	0.077	0.183	0.014
VIII	1.023	-0.001	0.151	0.014	-0.004	0.002	-0.108	0.134	1.842	-0.205	-0.288	-0.003
IX	0.217		0.039	0.001	0.020	-0.016	-0.008	0.011	0.373	0.004	0.004	-0.001
X	0.0034	-0.0019	-0.0022	-0.0109	-0.0187	-0.0201	-0.0372	0.0114	-0.0529	-0.1667	-0.0601	0.0001
XI	0.1826	-0.0010	0.0220	0.0021	0.0065	-0.0146	-0.0112	0.0179	0.2687	0.0036	0.0115	-0.0044
XII	0.4223	0.0110	0.0723	0.0143	0.0259	0.0055	-0.0041	0.0360	0.8106	0.0020	0.0077	0.0046
XIII	0.2070	0.0042	0.0358	0.0038	0.0086	-0.0181	0.0035	0.0195	0.3777	0.0073	-0.0174	-0.0038

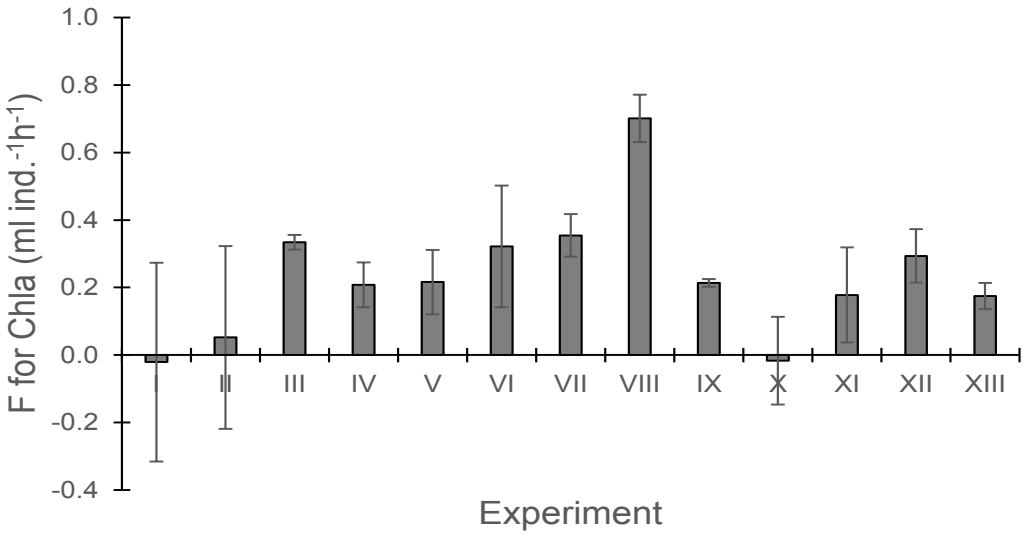


Figure 34 : *E. affinis* adults mean clearance rates (n=3 for 2013; n=6 for 2014 ± standard error in annexe 1) calculated from Chl *a* concentrations representing the total phytoplankton biomass. Vertical lines show standard deviation.

Clearance rates measured in term of Chl *a* concentrations ranged from -0.02 ±0.29 to 0.7 ±0.07 ml ind.$^{-1}$h^{-1} (Fig.34), and tented to be higher for Chl *a* concentrations between 1.5 and 3.8 µg L^{-1}.

E. affinis clearance rates calculated from fucoxanthin concentrations (representing diatoms), ranged from 0.02±0.24 to 0.81±0.07 ml ind.$^{-1}$h^{-1}. Clearance rates from alloxanthin concentrations, (representing cryptophyceae), and from lutein concentrations (representing chlorophyceae), were often negative, i.e. between -0.34±0.02 and 0.15±0.46 ml ind.$^{-1}$h^{-1}; -0.31±0.12 and 0.19±0.10 ml ind.$^{-1}$h^{-1}, respectively. The highest clearance rate from fucoxanthin values occurred at Chl *a* concentrations of 1.53 µg L^{-1} and, between 3.18 and 3.83 µg L^{-1} ($p<0.05$, Fig 35).

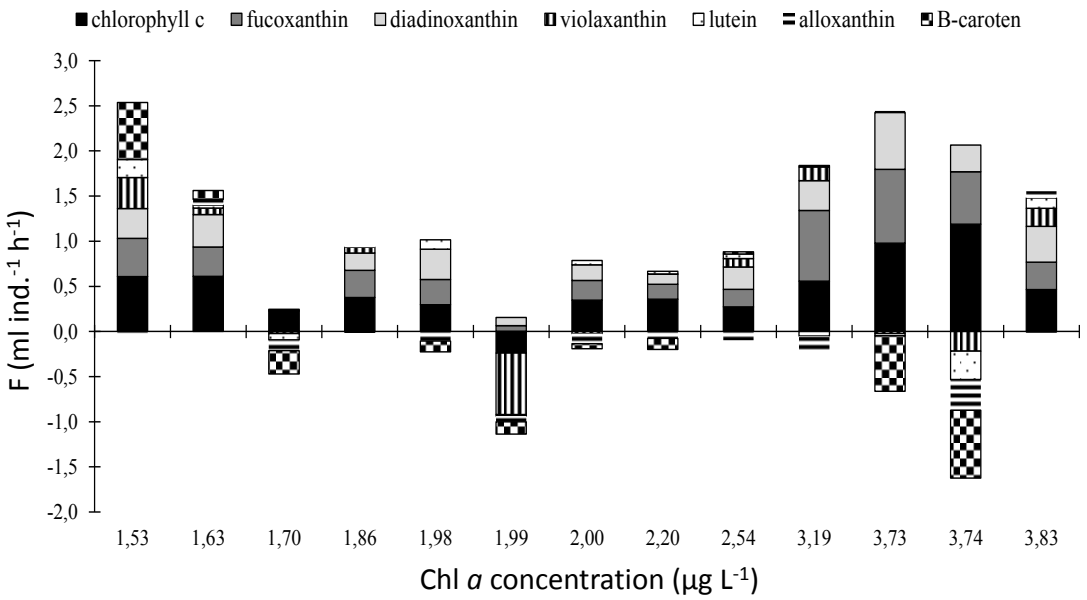


Figure 35 : ***E. affinis*** **adult mean clearance rates (n=3 for 2013; n=6 for 2014 ± standard error in annexe 1) measured from each biomarker pigment concentration for the grazing incubation experiments. X axis: Chl *a* concentration in water, at t_0 for each experiment.**

IV.2.4.4 Comparaison of ingestion rate results between both methods

In incubation experiments, the *E. affinis* ingestion rates calculated from Chl *a* concentrations varied from -0.08 ±0.29 to 1 ±0.07 ng ind.$^{-1}$ h^{-1} (Fig 36). Among the ingestion rates which were calculated from biomarker pigment concentrations, those calculated from fucoxanthin concentrations were generally the highest ($p<0.05$) excepted for two Chl *a* mean concentrations (3.18±0.40 and 3.8±0.51 µg L^{-1}).

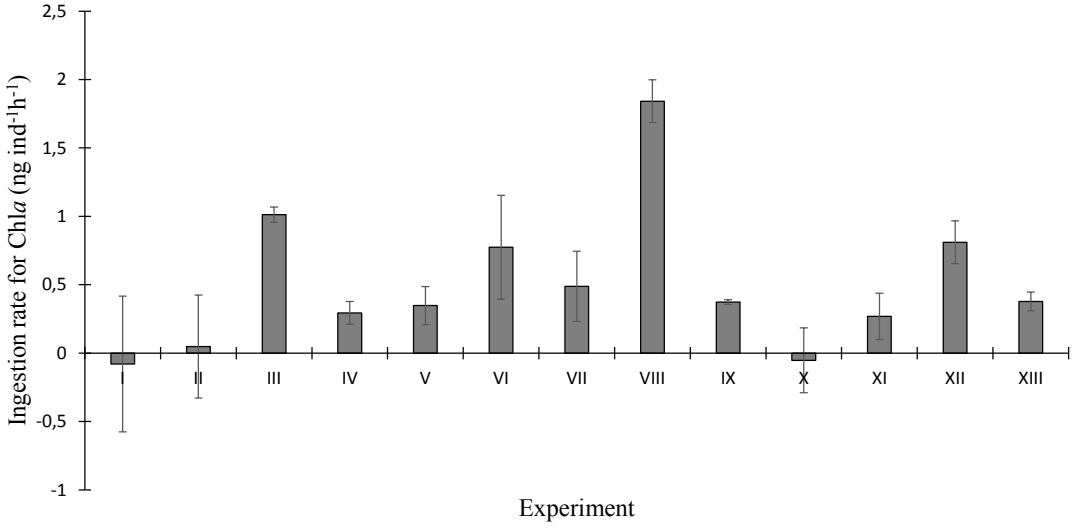


Figure 36 : ***E. affinis*** **adult mean Chl *a* ingestion rate n=3 for 2013; n=6 for 2014 ± standard error in annexe 1) (in all experiments. Vertical lines show standard deviation.**

In order to facilitate comparison of the results obtained by the two methods, the ingestion rates obtained from gut pigment content measurement of *E. affinis* are represented together with the ingestion rates from the incubation experiments (Fig. 37). Since only three biomarker pigments were quantified in the pigment gut contents; i.e. alloxanthin representing cryptophyceae, diatoxanthin representing diatoms, the comparison was made for these three markers. Pheophorbide *a* represented the ingestion of total biomass (Fig. 31b).

Mean ingestion rates calculated from pheaophorbide *a* concentrations in gut contents were not significantly different than the ingestion rates from Chl *a* concentrations calculated from the incubation experiments (p= 0.49). For gut content analyses, ingestion rates from alloxanthin concentrations ranged between 0.01 and 0.12 ng ind.$^{-1}$h^{-1} for Chl *a* concentrations comprised between 1.99 and 2.54 µg L^{-1} whereas they were negative for the incubation experiments. Ingestion rates from diatoxanthin concentrations were comprised between 0.03 and 0.08 ng ind.$^{-1}$h^{-1}.

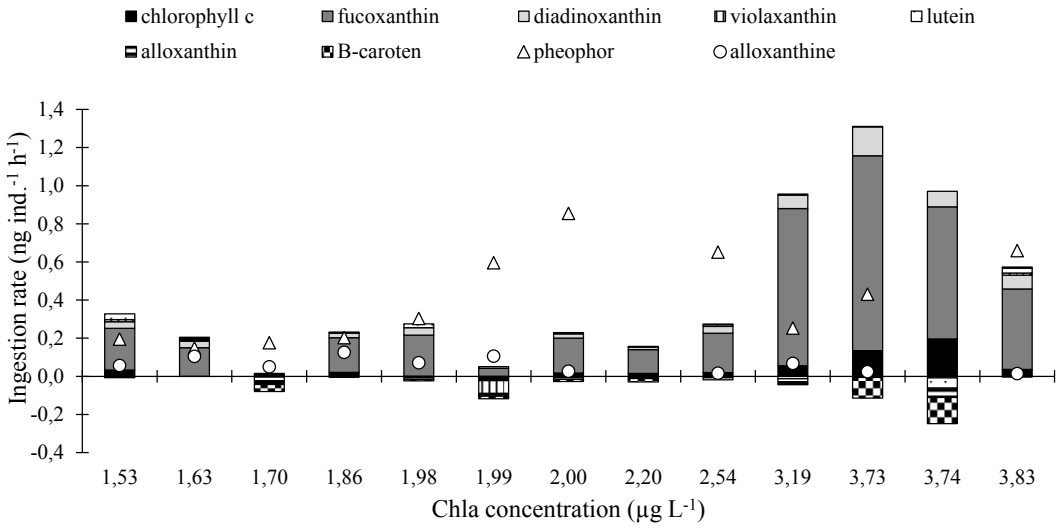


Figure 37 : *E. affinis* adult mean ingestion rates n=3 for 2013; n=6 for 2014 ± standard error in annexe 1 for incubations, and n=6 for 2013 and 2014 for gut contents calculated from the different pigment concentrations, obtained from incubation experiments (bars) and from gut content analyses (symbols). X axis: Chl *a* concentration, at t_0 of each experiment.

To detect any potential selective uptake of diatoms or cryptophyceae by the copepod *E. affinis*, the fucoxanthin / Chl *a* ingestion rate ratios (i.e. respective biomass proportions of diatoms and Chl *a* to total phytoplankton in the incubation experiments) were plotted against fucoxanthin / Chl *a*-eq concentration ratios (i.e. respective biomass proportions of diatoms and Chl *a*-eq to total phytoplankton in

copepod gut contents), calculated from the natural water used in the incubation experimentsat t_0 (Fig. 38a). All the values of the fucoxanthin /Chl *a*-eq ingestion rate ratios were systematically above the bisectrix of the graph representing the expected increase of these ingestion rate ratios as a function of fucoxanthin/Chl *a* concentration ratios when diatom ingestion by *E. affinis* is non selective. The same analysis was made for the alloxanthin / Chl *a* ratio obtained from ingestion rates calculated from gut content analyses and from the natural Scheldt water samples. Most ratios (7/10) from concentrations in the gut contents were also above the bisectrix (Fig. 38b). This shows that *E. affinis* consumed diatoms and cryptophyceae in disproportion to their abundance in the natural water, selecting them above other phytoplankton taxa.

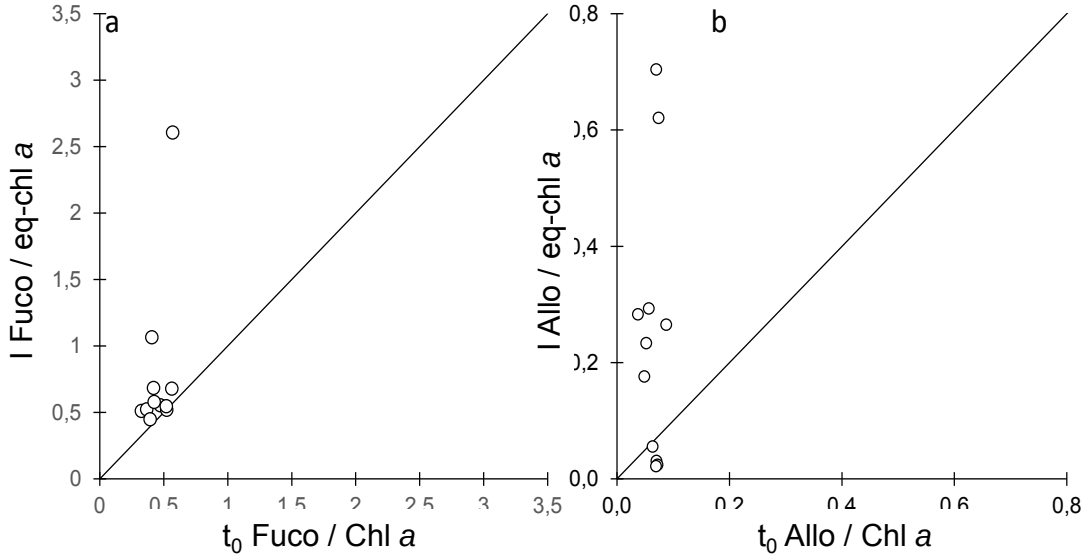


Figure 38 : Comparison of pigment proportions in *E. affinis* ingestion rate obtained from incubation experiments (a) and from gut contents (b) with the same proportions in the feeding medium.

IV.2.4.5 E. affinis impact on phytoplankton

Both methods used for *E. affinis* grazing quantification showed that, under natural feeding conditions, i.e. in the freshwater Scheldt, this species selected diatoms within the phytoplankton community. In the incubation experiments, ingestion rates of *E. affinis* were between 0.05 – 1.5 ng ind.$^{-1}$L^{-1}(mean around 0.6 ng ind.$^{-1}$h^{-1}) (Table 4). The 2013-2014 abundance of adults and CV *E. affinis* in the freshwater Scheldt in April was 6390±3521 ind. m^{-3} (Table 5). This means that the adult and CV *E. affinis* population removed 3.65% of the phytoplankton standing stock d^{-1}, and 2.06% of the diatom standing stock d^{-1}. *E. affinis* adult and CV dry weight (males and females averaged) was 10-14 µg ind^{-1} in 2010 (Lambert, 2012). Considering a mean value of

12 µg ind.$^{-1}$, the adults and CV daily ration (DR) in terms of Chl *a* / DW is 0.12 µgChl *a* µgDW d^{-1}. Applying the same DR to the younger development stages, the total consumption of phytoplankton stock by adults, copepodite and nauplii of *E. affinis* was 4,5 % d^{-1}.

Table 5 : *E. affinis* adult and CV abundance in April 2013-2014, and calculated grazing impact on the total phytoplankton and on the diatom stock.

	Adult + CV	Copepodites	Nauplii	Total population
Abundance april 2013-2014 (ind. m^{-3})	6390±3 521	1 596±401	18 733± 11 111	26719
Ingestion (µg L^{-1}d^{-1})	0.014	0.006	0.0006	0.0206
DW (µg ind.$^{-1}$)	12	5	0.5	
% of total phytoplancton consumed d^{-1}	3.65	0.39	0.46	4.5

IV.2.4.6 Microzooplancton experiments

For the total of the 4 experiments, only 16% of the pigments quantified showed a significant lower mean concentration in the microcosms at $t_{microzoo}$ than in the microcosm at t_c. These significant differences concerned concentrations of diatoxanthin (a diatom marker pigment), zeaxanthin (a chlorophyte marker pigment) and 2 pheopigments (pheophorbide *a*, and pheophytin *a*) in experiment 1, only pheophobide *a* in experiment 2, fucoxanthin (a diatom marker) and a violaxanthin (a chlorophyte pigment marker) in experiment 3. No significant grazing activity was detected in experiment 4. (Table 6). Consequently, the results of the microzooplankton grazing experiments were rather variable.

Table 6 : Results of the 4 grazing experiments with microzooplankton. Mean concentrations of each pigment (n=6 ± standard error in annexe2) at t_0, in control (tcontrol) and treatment (tzoo) microcosms. Mean clearance rates (F) and ingestion rates (I). (n=6± standard deviation in annexe 2).

Mean t_0 (µg L^{-1})												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
X	0.970	0.042	0.150	0.115	0.208	0.123	0.127	0.064	1.575	0.463	0.343	0.083
XI	1.022	0.034	0.178	0.112	0.167	0.102	0.165	0.070	1.870	0.465	0.245	0.056
XII	2.050	0.050	0.308	0.196	0.330	0.206	0.276	0.130	3.640	0.920	0.430	0.106
XIII	1.744	0.058	0.292	0.230	0.308	0.166	0.238	0.134	2.952	0.868	0.562	0.110

Mean tcontrol (µg L^{-1})												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
X	0.977	0.040	0.158	0.127	0.188	0.127	0.083	0.043	1.315	0.298	0.267	0.087
XI	1.102	0.035	0.182	0.110	0.168	0.107	0.110	0.072	1.918	0.367	0.237	0.058
XII	2.340	0.068	0.350	0.217	0.310	0.212	0.230	0.168	4.222	0.845	0.502	0.107
XIII	1.728	0.050	0.277	0.192	0.280	0.142	0.227	0.100	2.873	0.863	0.490	0.117

Mean tzoo (µg L^{-1})												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
X	0.875	0.035	0.117	0.123	0.178	0.113	0.070	0.063	1.392	0.233	0.267	0.092
XI	1.063	0.032	0.140	0.102	0.188	0.098	0.125	0.075	1.892	0.440	0.253	0.062
XII	1.917	0.072	0.303	0.187	0.283	0.152	0.212	0.143	3.873	0.767	0.425	0.117
XIII	1.553	0.046	0.250	0.162	0.260	0.132	0.203	0.103	2.753	0.678	0.360	0.098

Mean F (ml $L^{-1}h^{-1}$)												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
X	6.163	7.254	16.070	1.769	3.293	5.780	10.092	-19.002	-2.390	13.715	0.217	-2.495
XI	2.807	5.356	18.778	5.319	-5.028	7.883	-4.731	0.068	2.172	-7.893	-1.691	-2.069
XII	11.220	-1.212	8.660	8.694	5.604	19.150	7.671	11.005	5.659	5.457	10.217	-2.365
XIII	6.792	7.322	5.641	10.006	4.858	4.829	8.205	0.018	3.551	14.628	17.696	9.714

Mean I (ng $L^{-1}h^{-1}$)												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
X	5.108	0.258	2.042	0.159	0.533	0.663	0.849	-1.218	-4.213	3.761	0.003	-0.247
XI	1.600	0.248	2.485	0.580	-0.983	0.371	-1.565	-0.203	0.781	-4.273	-2.426	-0.173
XII	19.904	-0.335	2.197	1.434	1.392	2.981	1.041	0.971	16.192	4.132	3.556	-0.503
XIII	8.869	0.220	1.384	2.430	1.064	0.551	1.211	-0.186	6.153	8.970	7.074	0.896

Clearance rates measured varied from 0.02 – 19.15 ml L^{-1} h^{-1}, which means that, during the 24 h experiments, the microzooplancton grazed between 0.48 and 460.00 ml L^{-1} d^{-1} i.e. 4.8 to 46 % of certain phytoplankton taxa. No significant grazing on Chl *a* was detected, positive microzooplancton grazing was most frequently observed on pheopigments, this suggests that microzooplankton fed mainly on detritus.

IV.2.5 Discussion:

IV.2.5.1 SPM concentration and composition in the experimental water

In the water Scheldt water samples used for the grazing experiment, the SPM, in term of dry weight, was dominated by organic detritus, while live phytoplankton concentration, estimated from Chl *a* concentrations accounted for only a small share of SPM dry mass. The importance of OM in the SPM pool was evidenced by the significant correlation between OM and SPM, and the small fraction of live phytoplankton within this organic matter by the lack of such a correlation between Chl *a* and OM.

IV.2.5.2 Zooplankton feeding activity: methodological considerations

Despite the small contribution of live phytoplankton to estuarine SPM, several studies indicate a substantial herbivorous feeding mode of estuarine zooplankton (Gasparini et al., 1999; Calbet et al., 2000; Tackx et al., 2003). For this reason, and in view of the questions posed in the PhD work, we have opted for methods focusing on the quantification of zooplankton feeding on phytoplankton.

Numerous techniques exist to quantify grazing activity of pelagic or benthic organisms on phytoplankton. The use of radioactive markers (Haney, 1971, Daro, 1978) and later of stable isotope markers (e.g. Minagawa et Wada, 1984; Wada et al., 1991; Grey and Jones, 1999) and the quantification of pigments by fluorescence microscopy (Gasparini et Castel, 1997) or by quantification in gut content extracts (Mackas et Bohrer, 1976) were developed. Also, fatty acids as a food quality indicators and trophic tracers, can be analyzed in zooplankton (David et al., 2006; Gladyshev et al., 2016). More recently, the detection of trophic DNA has been developing quickly (e.g. Pommier et al., 2010).

The incubation method (Gauld, 1951) consists of creating a (preferably statistically significant) difference in concentration of the potential food items present in the medium between control and experimental microcosms and can in principle be adapted to all potential zooplankton preys which are quantifiable. This method was chosen for this study because of its feasibility in the field and in the laboratory conditions offered by EcoLab, ECOBE and LOG. An advantage of the incubation method is that it allows incubation of natural water samples, and hence the possibility to quantify zooplankton feeding activity and selectivity in quasi- natural circumstances (see further). Also, the incubation method allows to measure zooplankton grazing activity over relatively long periods, which is advantageous because most zooplankters are known have strong feeding rhythms (e.g. Daro, 1985; 1988 Sellner et al., 1994; Calliari et Antezana, 2001 ; Pagano et al., 2006).

A disadvantage of the incubation method is that, to arrive at significant food concentration differences between control and experimental bottles, the abundance of zooplankton has to be increased in comparison to the natural abundance of zooplankton in the field. For example, Lionard et al. (2005), did not find any significant grazing by *E. affinis* or on freshwater Scheldt phytoplankton in incubations using 20-40 *E. affinis* adults in a 1L volume of water during 24 h. In our experiments, 50 adult *E. affinis* were incubated in 900 mL of Scheldt water, whereas, field

abundance of *E. affinis* in the freshwater Scheldt seldom exceeded 7 individuals per liter. This concentrating of zooplankton can lead to a decrease in feeding activity measured, both with incubation time and with abundance of zooplankton organisms used (Roman et Rublee, 1980; Tackx et Polk, 1986, see production of particles).

These methods are also difficult and time consuming, especially when applied to zooplankton grazing experiments in natural water samples, which require working with several replicates because of natural variability of both the phytoplankton communities and the zooplankton feeding activity.

Besides the incubation method, we used the quantification of copepod gut pigment contents (Mackas et Bohrer, 1976). Pigments in copepod gut extracts were originally quantified by fluorimetry (e.g. Mackas et Boher, 1976; Gasparini et Castel, 1999) which allowed to quantify Chl *a* and its degradation pigments, and to calculate grazing on the total phytoplankton stock in the feeding medium. Measuring gut extracted pigments with HPLC, as applied in the Scheldt measurements, allows to quantify grazing activity on both the total phytoplankton community and on specific groups of phytoplankton, by the quantification of marker pigments, such as, for example, fucoxanthin for diatom and alloxanthin for cryptomonads (e.g. Everitt et al., 1990; Descy et al., 1999, Buffan –Dubau & Carman, 2000; Lemaire et al., 2002; Goffart, 2010; Oechsler-Christensen et al., 2011). A major advantage of gut pigment measurement is that the pigments are natural tracers of the feeding activity of the zooplankters in the field. However, the study of the pigment contents in the natural environment involves taking into account their degradation products. Besides abiotic degradation such as photo-oxydation, the pigments are subject to biological pressure that causes the same effects. These pressures are either natural cell senescence, or consumption by bacteria or even grazing by zooplankton. Many studies have shown the occurrence of these degradation pigments or pheopigments mainly for chlorophyll products (Head et al., 1992; Barlow et al., 1995; Cariou-LeGall et al., 1995; Llewelyn et al., 1996). Two groups of pheopigments are observed: chlorophyllide formed under the action of very active chlorophyllase during cell senescence and more particularly in some algal groups (diatoms) and pheophorbide associated with consumption activities by heterotrophic organisms. Carotenoids are degraded by the same processes, but the products are not necessarily identifiable (Descy et al., 1999, Antajan et Gasparini, 2004). Our HPLC analyses the phytoplankton taxa identified in

water of the Scheldt agree with previous reports of HPLC analysis in the freshwater Scheldt (Muylaert et al., 2000; Lionard et al., 2008).

We chose fucoxanthin pigment as diatom biomarker. The other diatom marker present in the samples was diadinoxanthin. The strong correlation between fucoxanthin and diadinoxantin in the ingestion rates calculated from the incubation experiments, (p-value = 0.002, Fig. 39) enforces the validity of the selection for diatoms deduced from our results.

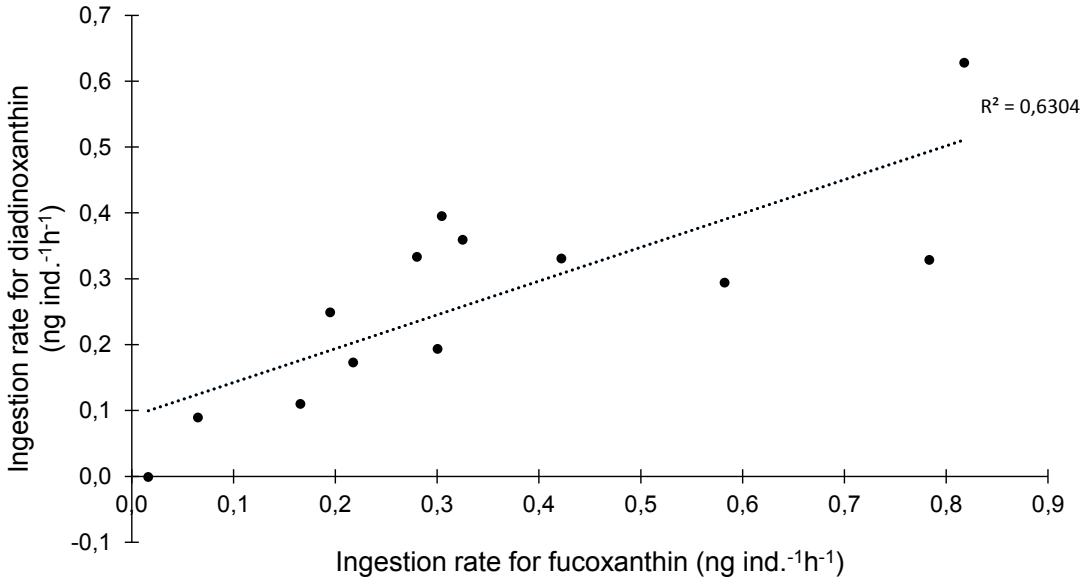


Figure 39 : Ingestion rate for diadinoxanthin as a function ingestion rate for fucoxanthin.

Ingestion rates calculated from marker pigments for chlorophytes and cryptomonads were much less significant and often negative. This aspect will be developped below.

IV.2.5.3 Clearance and ingestion rate:

To the best of our knowledge, this study provides the first application of HPLC quantification of phytoplankton biomass in zooplankton grazing experiments. The main advantage is that this allows to detect selective grazing activity on a certain type of algae, without necessarily having a significant concentration difference in Chl *a*-eq. concentration between control and experimental bottles (Leising et al., 2005). In our experiments, 32 % of the pigments quantified showed a significant difference in final concentration between control and experimental bottles. Fucoxanthin, the diatom pigment marker, revealed significant grazing activity in 54 % of the experiments with *E. affinis*. As in our experiments, diatoms were strongly

dominating the phytoplankton community, a significant grazing activity was also detected on Chl *a* concentration in 62 % of the experiments.

A surprising result is the absence from the guts of *E. affinis*. This constrasts with the significant ingestion rates calculated from diatom marker pigments for the incubation experiments. Indeed, according to the method which was used (i.e. incubation or gut content analysis) two different phytoplankton taxa were revealed as selected: diatoms for incubation experiment and cryptophyceae for gut content of *E. affinis*.

Pandolfini et al. (2000), showed that pigment processing during gut passage can be different between zooplankton species/groups (e.g. a copepod vs. a cladoceran) while Quiblier-Lloberas et al. (1994) and Oechsler-Christensen et al. (2011) did not find any significant differences between the zooplankton species tested. In *E. affinis* collected from the Scheldt, alloxanthin, was measured in the gut contents, but little or no other pigments. Similar results were found in two Wisconsin lakes the for copepods *Diaptomus minutus* and in lake of Esch-sur-Sûre for *Eudiaptomus gracilis* (Descy et al., 1999; Pandolfini et al., 2000).

IV.2.5.4 Negative clearance rates

For alloxanthin and lutein, negative clearance rates occurred in several incubation experiments. Production of algae in the grazing bottles as compared to the control bottles has been quite frequently reported in previous incubation experiments (e.g. Roman et Rublee, 1980; Tackx et Polk, 1986; Nejstgaard et al., 1997, 2001; Leising et al., 2005).

This phenomenon can, in principle, be caused by two processes:

1) Algal growth in the grazing bottles which can be stimulated by zooplankton excretion (Lehman, 1980; Roman et Rublee, 1980; Tackx et Polk, 1986)
2) Grazing pressure exerted on algae by microzooplancton is lessened in the grazing bottles because the mesozooplankton incubated feeds (also) on the microzooplancton (Nejstgaard et al., 2001),

IV.2.5.4.1 Algal growth in the grazing bottles can be stimulated by zooplankton excretion

For the occurrence of negative clearance rates in our experiments, the first explanation seems unlikely. Firstly, the experiments were run in the dark, which makes phytoplankton production unlikely. Yet, it could be possible that cells having stored enough light energy prior to the experiment, were stimulated in division by a nutrient boost (Vyverman, pers. comm.).

Total N excretion rates reported in literature for copepods the size of *E. affinis* are maximally around 1 µg N ind^{-1} day^{-1} (Saba et al., 2011, Alcaraz et al., 2013). Knowing that concentrations of NO_3-N, the most likely form of N to be rapidly taken up by the phytoplankton, in the Scheldt at the time of the experiments were around 4,8 µg ml^{-1}, the N addition by copepod excretion could amounts to maximally 1,25 %.

PO_4-P excretion rates reported in literature for copepods of the size of *E. affinis* are maximally 0,003 µg ind^{-1} day^{-1} (Saiz et Alcaraz, 1992; Alcaraz et al., 2013). In our experiments, 50 *E. affinis* adults were incubated during around 24 h in 900 ml of natural Scheldt water. This means that 0,15 µg -P was added in each bottle through copepod excretion bottle, i.e. 0,0002 µg ml^{-1}. In April 2013, PO_4-P concentration at Antwerp was minimally 0,1 µg ml^{-1}. So the increase in P concentration caused by copepod excretion in our experiments was maximally 0,2%.

Ning et al., 2013, report P excretion rates of a copepod / cladoceran community in Meiliang lake (China) of 0,20-0,57 mg P $(g\ DW)^{-1}$ h^{-1}. The mean DW of adult *E. affinis* (males and females) is 12 µg ind^{-1} (Lambert, 2012). So *E. affinis* P excretion during the 24 h incubation would have been be between 58 and 153 ng P ind^{-1} day^{-1}.The 50 *E. affinis* adults incubated would thus, have added , between 2,9 and 7,7µg P per bottle. This amounts to 0,003-0,009 µg P ml-1 which corresponds to a P concentration increase between 3-9 %.

Knowing that the PO4-P/N03-N ratio on a weight basis in the Upstream Scheldt in April 2013 was maximally 0,04, which is below the Redfield ratio of 0,08 (Redfield, 1934). P is probably the relative limiting nutrient. So, an increase of 9 % could have stimulated phytoplankton production. It should be realized however, that the P-excretion values reported by Ning et al, 2013, are for a mixed copepod/ cladoceran community, and are much higher than the values given by Saiz et Alcaraz (1992) and Alcaraz et al. (2013), which would not lead to a substantial P enrichment. As moreover the incubation took place in the dark, it is difficult to consider nutrient

enrichment, a major factor leading to phytoplankton production in the incubation experiment.

IV.2.5.4.2 Grazing pressure exerted on algae by microzooplancton can be lessened in the grazing bottles because the mesozooplankton incubated feeds (also) on the microzooplancton

Microzooplancton feeding on phytoplankton as the cause of negative clearance and ingestion rates occurring in our experiments was also possible. Microzooplankton is known to feed on rather small cells (e.g. Muylaert et al., 2000; 2005) and in our experiments, negative clearance rates occured mainly for alloxanthin and lutein, marker pigments for cryptophyceae and green algae, which are, generally, smaller than most diatoms.

It should be realized that feeding by the mesozooplankton organism incubated on microzooplancton happens not only in the case that negative clearance rates were observed at the end of the experiments, but can also lead to an underestimation of (positive) clearance rates calculated for the mesozooplankter studied. Reducing the microzooplancton predation pressure on phytoplankton in the grazing bottles versus the control bottles means that the average phytoplankton concentration in the grazing bottles during the experiment is higher than calculated by comparison with the control bottles at the end of the experiment following Frost (1972).

Muylaert et al. (2000), report the existence of substantial populations of heterotrophic nannoflagellates (HNAN) in the freshwater Scheldt estuary. Microscopic analysis showed most flagellates containing pigments to belong to autotrophic or weakly heterotrophic taxa, which led Muylaert et al., 2000 to conclude that most HNAN in the freshwater Scheldt are bacterivorous rather than algivorous. Also ciliates, among which oligotrichs, known to feed on bacteria, HNAN and small algae, were observed in the upstream Scheldt in the Muylaert et al. (2000) study. Lionard et al. (2005), quantified microzooplancton grazing on the spring phytoplankton community in the upper Scheldt estuary by comparing algal pigment concentrations in an incubation experiment using natural Scheldt water filtered through 20 µm (control) and 200µm (with microzooplancton). They report that the 20-200 µm fraction contained mainly rotifers, and that grazing impact of the microzooplancton could remove between 33 and 84 % of the phytoplankton standing stock day^{-1}. Grazing by rotifers did not show a selectivity for any algal groups.

In the 2014 experiments run to quantify microzooplancton grazing activity, microzooplancton community clearance rates measured varied from 0.06 - 19 ml L^{-1} h^{-1}, which means that, during the 24 h experiments, the microzooplancton grazed between 1.44 and 456 ml L^{-1} d^{-1} i.e. 0.144 to 45, 60 % of certain phytoplankton taxa. These values are in the same range as those reported by Lionard et al. (2005) (33-84 % d^{-1}), but their results consider the microzooplankton grazing pressure on the total phytoplankton community. In our experiments, no significant grazing on Chl *a*, representing the total phytoplankton biomass, was ever detected. As positive microzooplancton grazing was most frequently observed on pheopigments, this suggests that microzooplankton fed mainly on detritus.

For mesozooplankton predation on microzooplankton to have a substantial impact on the estimation of mesozooplankton grazing on phytoplankton, the former has to be substantial. However, in most estuarine feeding experiments, *E. affinis* is shown to be mostly herbivorous. Fatty acid analysis carried out on *E. affinis* females collected from the 0.5-1.8 salinity range in the Scheldt during 2007-2008 suggested a dominant contribution of phytoplankton versus terrestrial or heterotrophic inputs in *E. affinis'* diet (Mialet et al., unpublished). Another fatty acid analysis performed on *E. affinis* sampled in 2005 for the same season and area (Van den Meersche et al., 2009) reflected a slightly more carnivorous but still phytoplankton dominated regime for *E. affinis*. Also in the St Laurence estuary, fatty acid analysis have shown that both the North Atlantic of *E. affinis* clade, living in the brackish waters and the Atlantic clade, living in the lower salinity range of the estuary feed mainly on phytoplankton (Cabrol et al., 2015; Pommier et al., 2010). This suggests an efficient transfer of diatom fatty acids to *E. affinis* in this estuary. Yet, *E. affinis* is known to be able of carnivorous feeding. In the Gironde estuary, where SPM is even very heavily loaded with inorganic and highly degraded organic material, Gasparini and Castel (1997) observe that E. *affinis* switches to heterotrophic preys at high SPM concentrations, but that nannoplankton remains its bulk food. In the same estuary, David et al. (2006), observe that *E. affinis* herbivorous feeding does not cover its energy requirements and suggest it supplements these with protozoa.

IV.2.5.5 Comparison with other studies

In our experiments, the ingestion rates obtained in terms of Chl *a* ranged from 0,05-1,84 ng ind.$^{-1}$h^{-1}, with an average of 0,64 ±0,50 ng ind.$^{-1}$h^{-1}. Taking into account a GCR of 1,99 h^{-1} (see material and methods), this amounts to a gut content of 0,32 ± 0,25 ng ind.$^{-1}$. Gut pigment content values for pheophorbide were between 0,15 and 0,86, with a mean value of 0,41 ± 0,25 ng ind.$^{-1}$. So both methods applied yielded similar ingestion rates for Chl *a*. Oechsler-Christensen et al. (2011), compared ingestion rates for different pigments from gut pigment contents of 3 marine copepods with results from incubation experiments using microscopic cell counts to quantify grazing selectivity, and also found that the two methods gave similar results. Mialet et al. (2010), measured Chl *a*–eq pigment contents of 0,67 ± 0,57 ng ind^{-1} for *E. affinis* adults in the brackish –freshwater fringe of the Scheldt. Gasparini et al. (1999) and Tackx et al. (2003) observed *E. affinis* Chl *a* –eq gut contents of 1,03 ± 0,38 ng ind.$^{-1}$ for *E. affinis* females in the brackish reach of the Scheldt. So the values obtained in this study are comparable to those of Mialet et al. (2010).

Because of little available experimental data on estuarine zooplankton feeding, we have compared the Chl *a*-eq. gut contents measured in our study with those of copepods of similar size in various environments in order to situate our results.

Table 7 : Comparison of gut pigment content between different copepod taxa.

Copepod species	Size (µm)	Individual dry weight (µg ind.$^{-1}$)	Gut content (ng Chla ind.$^{-1}$)	Environment	Source
Eurytemora affinis	**800 - 1000**	**09 -12**	**0,15-0,86**	**Freshwater estuary**	**This study**
Pseudodiaptomus hessei		8.4 -16.39	0,16-1,63	Sundays River estuary	Jerling & Wooldridge 1991 ; Kibirige & Perissinotto 2003
Acartia natalensis		1.65	0,12-0,45	Sundays River estuary	Kibirige & Perissinotto 2003 ; Wooldridge & Bailey, 2015
Pseudocalanus sp			0.1 - 0.75	Oresund	Nicolajsen et al., 1983
Centropages hamatus			0.1 - 0.8	Oresund	Nicolajsen et al., 1983
Calanus helgolandicus	3800-3900	265,04	0.09-0.28	Celtic sea	Williams & Robins, 1982
Centropages typicus	1600 - 2000	23,2 - 50,8 (female)	0.3-1.9 (female)	New York shelf waters / New york Bight	Dagg et Grill, 1980Smith & Lane, 1987
	890-1120	23.2–41.8 (female)	0.3-1.9 (female)	New York shelf waters / New york Bight	Dagg et Grill, 1980Smith & Lane, 1987
Temora longicornis	990 - 1300		0.1-1.4	Marine plankton	Wang , 1986 ; Dam et Peterson, 1991
Acartia tonsa	91 µm	7.97 (female)	0.06 -0.19	Marine plankton / Narragansett Bay, Rhode Island	Durbin & Durbin, 1981 ; Kiorboeet Tiselius, 1987
	740 µm	6.03 (male)		Narragansett Bay Rhode Island	Durbin & Durbin, 1981

As can be seen form table 7, our results on gut pigment contents for *E. affinis* fall within the range reported for other copepod species. In our study, measurements of *E. affinis* grazing activity on total phytoplankton by both methods gives statistically the same results. However, both these measurements are considerably lower than the *E. affinis* gut pigment content values measured in the Scheldt brackish water zone by Gasparini et al. (1999) and Tackx et al. (2005). This could be due to the fact that the measurements in brackish water only considered female *E. affinis*, which are known

to feed at a higher rate than males and also, that Chl *a* –eq pigment gut contents by Gasparini et al. (1999), also used in Tackx et al. (2005), were in part made by a fluorimeter, which probably overestimates in comparison to HPLC.

IV.2.5.6 Impact of E. affinis grazing on phytoplankton

Our results show that the impact of *E. affinis* grazing on the total spring phytoplankton population was very low. The low grazing impact of *E. affinis* on the phytoplankton could also explain why Lionard et al. (2005), using relatively low abundance of animals in their incubation experiments did not measure a significant impact of adult and CV copepods (*E. affinis* and cyclopoids) on the Scheldt phytoplankton.

To verify the realism of our results, we converted the µg Chl *a* – ingestion rates to carbon ingestion rates, using a Chl *a* –C conversion of min 10 and max 30 (Lionard et al., 2008; PAE- OMES unpublished results) and for zooplankton, and a DW-C conversion of 45 %. A mean ingestion rate of 0,6 ng Chl *a* ind^{-1} h^{-1} for *E. affinis* adults and CV, with a mean DW of 12 µg ind^{-1}; corresponds to 0.14-0.42 µg C /5,4 µg C d^{-1}; i.e. a daily ration (DR) of 2.5-7.8 %.

These values fall within the range of Daily Ration values found in the literature for copepods (e.g. Calbet et al., 2000).

The extrapolation of the DR calculated for adults and V stages to copepodite and nauplii might represent an underestimation of the population impact, as younger stages, which except for N1-2, usually feed at a higher rate than adults.

As the phytoplankton is strongly dominated by diatoms, grazing pressure on these is also low. So it is unlikely that *E. affinis* will overexploit the diatom stock, nor that the development of *E. affinis* is limited by phytoplankton food availability. While cryptomonads appeared ingested in some of the gut pigment contents showing alloxanthin, this pigment was produced in the experimental bottles as compared to the controls in several incubation experiments. The impact calculated from the gut content derived alloxanthin ingestion rates is <1% d^{-1}, which renders it unlikely that strong grazing pressure is exerted by *E. affinis* on cryptophytes. Lutein, a marker for chlorophytes, was never found in the gut, and was often produced in the experimental incubation bottles. As we have shown above, nutrient enrichment is unlikely as a

cause of the stimulated production of some phytoplankton taxa in the experimental bottles.

So, even the fact that phytoplankton ingestion measures in this study seems to satisfy energetic requirements of *E. affinis*, and that both the methods used give comparable results, which are confirmed by the literature comparison, it seems worthwhile to verify if any *E. affinis* feeding activity on microzooplancton occurs in Scheldt water in future incubation experiments.

The results of the microzooplankton experiment also suggest the need for follow up, as the measure clearance rates on the phytoplankton community are sometimes substantial (up to 19 ml $L^{-1}h^{-1}$). The problem is that the results of these experiments were very variable: the pigments on which a significant effect by microzooplankton feeding was measured, were variable from one experiment to the other, and in one experiment, no effect was detected at all. In this work, we only conducted 4 microzooplankton experminents, so clearly, more are needed to evaluate the trophic role of microzooplankton in the Scheldt.

While this method allowed to distinguish taxonomic groups selected by *E. affinis*, or the microzooplankton community, it is not suffciently precise to detect if the change occurring in the phytoplankton community (an increase in small diatom taxa (*Cyclotella* spp.) and a decrease in the big diatom species (*Actinocyclus normannii*) is caused by *E. affinis* selective grazing. Several studies have shown that HPLC analysis could be associated with optical microscopy to identify the dominant species (Havskum et al., 2004; Lampert, 2001). A comparative study by Roy et al. (1996) showed than data provided by microscopy allow to go beyond the HPLC in the specific determination except for very small cells.

During our experimentation, 100 ml samples of all bottles were fixed with formalin and kept for microscopic analysis. This work is presently being carried out as part of Master thesis, as it was too time consuming to be realized within the time limit of this thesis.

IV.2.6 References

A --

Alcaraz, M., Almeda, R., Saiz, E., Calbet, A., Duarte, C. M., Agustí, S., & Alonso, A. (2013). Effects of temperature on the metabolic stoichiometry of Arctic zooplankton. Biogeosciences, 10689-697.

Antajan, E., & Gasparini, S. (2004). Assessment of Cryptophyceae ingestion by copepods using alloxanthin pigment: a caution. Marine Ecology Progress Series, 274, 191-198.

Appeltans, W., Hannouti, A., Van Damme, S., Soetaert, K., Vanthomme, R. & Tackx, M., 2003. Zooplankton in the Schelde estuary (Belgium/The Netherlands). The distribution of *Eurytemora affinis*: effect of oxygen? Journal of Plankton Research 25, 1441-1445.

B --

Barlow, R. G., Mantoura, R. F. C., Cummings, D. G., & Fileman, T. W. (1997). Pigment chemotaxonomic distributions of phytoplankton during summer in the western Mediterranean. Deep Sea Research Part II: Topical Studies in Oceanography, 44(3), 833-850.

Buffan-Dubau, E., & Carman, K. R. (2000a). Diel feeding behavior of meiofauna and their relationships with microalgal resources. Limnology and Oceanography, 45(2), 381-395.

Bodineau, L., Thoumelin, G., Béghin, V., & Wartel, M. (1998). Tidal time-scale changes in the composition of particulate organic matter within the estuarine turbidity maximum zone in the macrotidal Seine Estuary, France: the use of fatty acid and sterol biomarkers. Estuarine, Coastal and Shelf Science, 47(1), 37-49.

C --

Cabrol, J., Winkler, G., & Tremblay, R. (2015). Physiological condition and differential feeding behaviour in the cryptic species complex *Eurytemora affinis* in the St Lawrence estuary. Journal of Plankton Research, fbu111.

Calbet, A., Trepat, I., & Arin, L. (2000). Naupliar growth versus egg production in the calanoid copepod *Centropages typicus*. Journal of plankton research, 22(7), 1393-1402.

Calliari, D., & Antezana, T. (2001). Diel feeding rhythm of copepod size-fractions from Coliumo Bay, Central Chile. Scientia Marina, 65(4), 269-274.

Cox, T.J.S., Maris, T., Soetaert, K., Conley, D.J., Van Damme, S., Meire, P., Middelburg, J.J., Vos, M. & Struyf, E., 2009. A macro-tidal freshwater ecosystem recovering from hypereutrophication: the Schelde case study. Biogeosciences 6, 2935-2948.

D --

Daro, M. H. (1978). A simplified14C method for grazing measurements on natural planktonic populations. Helgoländer wissenschaftliche Meeresuntersuchungen, 31(1-2), 241-248.

Daro, M. H. (1985). Feeding rhythms and vertical distribution of marine copepods. Bulletin of marine science, 37(2), 487-497.

Daro, M. H. (1988). Migratory and grazing behavior of copepods and vertical distribution of phytoplankton. Bulletin of Marine Science, 43(3), 710-729.

David, V., Sautour, B., Galois, R., & Chardy, P. (2006). The paradox high zooplankton biomass–low vegetal particulate organic matter in high turbidity zones: What way for energy transfer? Journal of Experimental Marine Biology and Ecology, 333(2), 202-218.

DeMott, W. R. (1988). Discrimination between algae and detritus by freshwater and marine zooplankton. Bulletin of Marine Science, 43(3), 486-499.

Descy, J. P., Frost, T. M., & Hurley, J. P. (1999). Assessment of grazing by the freshwater copepod *Diaptomus minutus* using carotenoid pigments: a caution. Journal of Plankton Research, 21(1), 127-145.

Durbin, E.G. & Durbin, E.G., 1992. Effects of temperature and food abundance on grazing and short-term weight change in the marine copepod *Acartia hudsonica*. Limnoogy and Oceanography, 37: 361-378.

Dutz, J., Koski, M., & Jónasdóttir, S. H. (2008). Copepod reproduction is unaffected by diatom aldehydes or lipid composition. Limnology and Oceanography, 53(1), 225.

E, F --

Elliott, M., & Quintino, V. (2007). The estuarine quality paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. Marine Pollution Bulletin, 54(6), 640-645.

Everitt, D. A., Wright, S. W., Volkman, J. K., Thomas, D. P., & Lindstrom, E. J. (1990). Phytoplankton community compositions in the western equatorial Pacific determined from chlorophyll and carotenoid pigment distributions. Deep Sea Research Part A. Oceanographic Research Papers, 37(6), 975-997.

Frost, B. W. (1972). Effects of size and concentration of food particles on the feeding behavior of the marine plankton copepod *Calanus pacificus*. Limnology and oceanography, 17(6), 805-815.

G --

Gall, C. L., & Blanchard, G. (1995). Monthly HPLC measurements of pigment concentration from an intertidal muddy sediment of Marennes-Oléron Bay, France. Marine Ecology Progress Series, 121(1-3), 171-179.

Gasparini, S., & Castel, J. (1997). Autotrophic and heterotrophic nanoplankton in the diet of the estuarine copepods *Eurytemora affin*is and *Acartia bifilosa*. Journal of Plankton Research, 19(7), 877-890.

Gasparini, S., Castel, J., & Irigoien, X. (1999). Impact of suspended particulate matter on egg production of the estuarine copepod, *Eurytemora affinis*. Journal of Marine Systems, 22(2), 195-205.

Gauld, D. T. (1951). The grazing rate of planktonic copepods. Journal of the Marine Biological Association of the United Kingdom, 29(03), 695-706.

Gladyshef, M., Makhutova, O., Kravchuk, E., Anishchenko, O., & Sushchik, N., (2016). Stable isotope fractionation of fatty acids of *Daphnia* fed laboratory cultures of microalgae. Limnologica 56, 23–29

Goffart, A. (2010). Mise au point de l'indice composition dans le cadre de l'indicateur phytoplancton-les indices de composition phytoplanctonique en eaux côtières: synthèse bibliographique. IFREMER.

Grey, J., & Jones, R. I. (1999). Carbon stable isotopes reveal complex trophic interactions in lake plankton. Rapid Communications in Mass Spectrometry, 13(13), 1311-1314.

H --

Havskum, H., Schlüter, L., Scharek, R., Berdalet, E., & Jacquet, S. (2004). Routine quantification of phytoplankton groups-microscopy or pigment analyses? Marine Ecology Progress Series, 273, 31-42.

Hart, R. C. (1988). Zooplankton feeding rates in relation to suspended sediment content: potential influences on community structure in a turbid reservoir. Freshwater Biology, 19(1), 123-139.

Haney, R. L. (1971). Surface thermal boundary condition for ocean circulation models. Journal of Physical Oceanography, 1(4), 241-248.

Head, E. J. H., & Harris, L. R. (1992). Chlorophyll and carotenoid transformation and destruction by *Calanus* spp. grazing on diatoms. Marine Ecology-Progress Series, 86, 229-229.

Heip, C., Warwick, R. M., Carr, M. R., Herman, P. M., Huys, R., Smol, N., & Van Holsbeke, K. (1988). Analysis of community attributes of the benthic meiofauna of Frierfjord/Langesundfjord. Marine Ecology Progress Series, 46, 171-180.

Heinle, D. R., Harris, R. P., Ustach, J. F., & Flemer, D. A. (1977). Detritus as food for estuarine copepods. Marine Biology, 40(4), 341-353.

I, J --

Irigoien, X., Head, R. N., Harris, R. P., Cummings, D., Harbour, D., & Meyer-Harms, B. (2000). Feeding selectivity and egg production of *Calanus helgolandicus* in the English Channel. Limnology and Oceanography, 45(1), 44-54.

Jack, F. R., Paterson, A., & Piggott, J. R. (1993). Relationships between rheology and composition of Cheddar cheeses and texture as perceived by consumers. International journal of food science & technology, 28(3), 293-302.

Jeffrey, S. W., & Mantoura, R. F. C. (1997). Development of pigment methods for oceanography: SCOR-supported working groups and objectives. Na.

Jónasdóttir, S. H., Dutz, J., Koski, M., Yebra, L., Jakobsen, H. H., Vidoudez, C. & Nejstgaard, J. C. (2011). Extensive cross-disciplinary analysis of biological and chemical control of *Calanus finmarchicus* reproduction during an aldehyde forming diatom bloom in mesocosms. Marine biology, 158(9), 1943-1963.

K, L --

Kagata, H., & Ohgushi, T. (2011). Ecosystem consequences of selective feeding of an insect herbivore: palatability–decomposability relationship revisited. Ecological Entomology, 36(6), 768-775.

Kirk, K. L., & Gilbert, J. J. (1990). Suspended clay and the population dynamics of planktonic rotifers and cladocerans. Ecology, 1741-1755.

Lambert, C. (2012). Etude des caracteristiques morphometrique d'*Eurytemora affinis* (Poppe, 1880, copepodes, calanoïdes) comme indicatrices de qualité du milieu. Rapport Stage M1 Ecologie Université Paul sabatier. 26pp.

Lampert, L. (2001). Dynamique saisonnière et variabilité pigmentaire des populations phytoplanctoniques dans l'Atlantique Nord (Golfe de Gascogne) (Doctoral dissertation, Brest).

Lehman, J. T. (1980). Release and cycling of nutrients between planktonic algae and herbivores. Limnology and Oceanography, 25(4), 620-632.

Leising, A. W., Pierson, J. J., Halsband-Lenk, C., Horner, R., & Postel, J. (2005). Copepod grazing during spring blooms: can Pseudocalanus newmani induce trophic cascades?. Progress in Oceanography, 67(3), 406-421.

Lionard, M., Azémar, F., Boulêtreau, S., Muylaert, K., Tackx, M., & Vyverman, W. (2005). Grazing by meso-and microzooplankton on phytoplankton in the upper reaches of the Schelde estuary (Belgium/The Netherlands). Estuarine, Coastal and Shelf Science, 64(4), 764-774.

Lionard, M., Muylaert, K., Van Gansbeke, D., & Vyverman, W. (2005). Influence of changes in salinity and light intensity on growth of phytoplankton communities from the Schelde river and estuary (Belgium/The Netherlands). Hydrobiologia, 540(1-3), 105-115.

Lionard, M., Muylaert, K., Tackx, M., & Vyverman, W. (2008). Evaluation of the performance of HPLC–CHEMTAX analysis for determining phytoplankton biomass and composition in a turbid estuary (Schelde, Belgium). Estuarine, Coastal and Shelf Science, 76(4), 809-817.

Llewelyn, R. V., & Williams, J. R. (1996). Nonparametric analysis of technical, pure technical, and scale efficiencies for food crop production in East Java, Indonesia. Agricultural Economics, 15(2), 113-126.

Löder, M. G., Meunier, C., Wiltshire, K. H., Boersma, M., & Aberle, N. (2011). The role of ciliates, heterotrophic dinoflagellates and copepods in structuring spring plankton communities at Helgoland Roads, North Sea. Marine biology, 158(7), 1551-1580.

M --

Mackas, D., & Bohrer, R. (1976). Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. Journal of experimental marine biology and ecology, 25(1), 77-85.

Maes, J., Tackx M. & K. Soetaert, K., (2005). The predation impact of juvenile herring *Clupea harengus* and sprat *Sprattus sprattus* on estuarine zooplankton. Hydrobiologia (2005) 540, 225–235.

Matsuno, T. (1989). Animal carotenoids. In *Carotenoids* (pp. 59-74). Springer US.

Martel, C. M. (2006). Prey location, recognition and ingestion by the phagotrophic marine dinoflagellate *Oxyrrhis marina*. Journal of Experimental Marine Biology and Ecology, 335(2), 210-220.

Meire, P., Ysebaert, T., Van Damme, S., Van den Bergh, E., Maris, T. & Struyf, E., (2005). The Scheldt estuary: a description of a changing ecosystem. *Hydrobiologia 540(1)*, 1-11.

Mialet, B., Azémar, F., Maris, T., Sossou, C., Ruiz, P., Lionard, M., Van Damme, S., Lecerf, A., Muylaert, K., Toumi, N., & Tackx, M. (2010). Spatial spring distribution of the copepod *Eurytemora affinis* (Copepoda, Calanoida) in a restoring estuary, the Scheldt (Belgium), Estuarine, Coastal and Shelf Science, 88, 116-124.

Mialet, B., Gouzou, J., Azémar, F., Maris, T., Sossou, C., Toumi, N., Van Damme, S., Meire, P., & Tackx, M. (2011). Response of zooplankton to improving water quality in the Scheldt estuary (Belgium). *Estuarine, Coastal and Shelf Science 93*, 47-57.

Mialet, B., Majdi, N., Tackx, M., Azémar, F., & Buffan-Dubau, E. (2013). Selective feeding of bdelloid rotifers in river biofilms. PloS one, 8(9), e75352.

Minagawa, M., & Wada, E. (1984). Stepwise enrichment of 15 N along food chains: further evidence and the relation between δ 15 N and animal age. Geochimica et cosmochimica acta, 48(5), 1135-1140.

Miquelis, A. (1996). Facteurs de contrôle des populations zooplanctoniques en milieu fluvial (la Seine), relations fleuve-annexes hydrauliques (Doctoral dissertation).

Miralto, A., Barone, G., Romano, G., Poulet, S. A., Ianora, A., Russo, G. L., & Giacobbe, M. G. (1999). The insidious effect of diatoms on copepod reproduction. Nature, 402(6758), 173-176.

Muylaert, K., Sabbe, K., & Vyverman, W. (2000). Spatial and temporal dynamics of phytoplankton communities in a freshwater tidal estuary (Schelde, Belgium). Estuarine, Coastal and Shelf Science, 50(5), 673-687.

Muylaert, K., Tackx, M., & Vyverman, W. (2005). Phytoplankton growth rates in the freshwater tidal reaches of the Schelde estuary (Belgium) estimated using a simple light-limited primary production model. Hydrobiologia, 540(1-3), 127-140.

Nejstgaard, J. C., Gismervikz, I., & Solberg, P. T. (1997). Emiliania huxleyi. Marine Ecology Progress Series, 147, 197-217.

N, O --

Nejstgaard, J. C., Naustvoll, L. J., & Sazhin, A. (2001). Correcting for underestimation of microzooplankton grazing in bottle incubation experiments with mesozooplankton.

Oechsler-Christensen, B., Jónasdóttir, S. H., Henriksen, P., & Hansen, P. J. (2011). Use of phytoplankton pigments in estimating food selection of three marine copepods. Journal of plankton research, fbr090.

Paffenhöfer, G. A., & Van Sant, K. B. (1985). The feeding response of a marine planktonic copepod to quantity and quality of particles. Mar Ecol Prog Ser, 27, 55-65.

P--

Pandolfini, E., Thys, I., Leporcq, B., & Descy, J. P. (2000). Grazing experiments with two freshwater zooplankters: fate of chlorophyll and carotenoid pigments. Journal of Plankton Research, 22(2), 305-319.

Pagano, M., Champalbert, G., Aka, M., Kouassi, E., Arfi, R., Got, P. & Bouvy, M. (2006). Herbivorous and microbial grazing pathways of metazooplankton in the Senegal River Estuary (West Africa). Estuarine, Coastal and Shelf Science, 67(3), 369-381.

Pasternak, A. F., & Schnack-Schiel, S. B. (2001). Feeding patterns of dominant Antarctic copepods: an interplay of diapause, selectivity, and availability of food. In Copepoda: Developments in Ecology, Biology and Systematics (pp. 25-36). Springer Netherlands.

Pommier, T., Neal, P. R., Gasol, J. M., Coll, M., Acinas, S. G., & Pedrós-Alió, C. (2010). Spatial patterns of bacterial richness and evenness in the NW Mediterranean Sea explored by pyrosequencing of the 16S rRNA. Marine Ecology Progress Series, 400,143–154.

Poulet, S. A., & Ouellet, G. (1982). The role of amino acids in the chemosensory swarming and feeding of marine copepods. Journal of Plankton Research, 4(2), 341-361.

Q

Quiblier, C., Bourdier, G., Amblard, C., & Pepin, D. (1994). Separation of phytoplanktonic pigments by HPLC for the study of phyto-zooplankton trophic relationships. Aquatic Sciences-Research Across Boundaries, 56(1), 29-39.

Quiblier-Lloberas, C., Bourdier, G., Amblard, C., & Pepin, D. (1996). A qualitative study of zooplankton grazing in an oligo-mesotrophic lake using phytoplanktonic pigments as organic markers. Limnology and Oceanography, 41(8), 1767-1779.

R

Redfield, A. C. (1934). On the proportions of organic derivations in seawater and their relation to the composition of plankton (reprint). Benchmark Papers in Ecology, 1.

Roman, M. R., & Rublee, P. A. (1980). Containment effects in copepod grazing experiments: a plea to end the black box approach. Limnology and Oceanography, 25(6), 982-990.

Roy, S., Chanut, J.P., Gosselin, M., & Sime-Ngando, T. (1996). Characterization of phytoplankton communities in the lower St. Lawrence Estuary using HPLC-detected pigments and cell microscopy. Mar Ecol Prog Ser, 142, 55-73.

S

Saba, G. K., Steinberg, D. K., & Bronk, D. A. (2011). The relative importance of sloppy feeding, excretion, and fecal pellet leaching in the release of dissolved carbon and nitrogen by *Acartia tonsa* copepods. Journal of Experimental Marine Biology and Ecology, 404(1), 47-56.

Saiz, E., Alcaraz, M., & Paffenhöfer, G. A. (1992). Effects of small-scale turbulence on feeding rate and gross-growth efficiency of three *Acartia* species (Copepoda: Calanoida). Journal of Plankton Research, 14(8), 1085-1097.

Sellner, K. G., Olson, M. M., & Kononen, K. (1994). Copepod grazing in a summer cyanobacteria bloom in the Gulf of Finland. In Ecology and Morphology of Copepods (pp. 249-254). Springer Netherlands.

Steele, J. H. (1974). The structure of marine ecosystems.

T --

Tackx, M., & Polk, P. (1986). Effect of incubation time and concentration of animals in grazing experiments using a narrow size range of particles. IZWO Collected Reprints.

Tackx, M., Irigoien, X., Daro, N., Castel, J., Zhu, L., Zhang, X., & Nijs, J. (1995). Copepod feeding in the Westerschelde and the Gironde. Hydrobiologia, 311(1), 71-83.

Tackx, M. L. M., Herman, P. J. M., Gasparini, S., Irigoien, X., Billiones, R., & Daro, M. H. (2003). Selective feeding of *Eurytemora affinis* (Copepoda, Calanoida) in temperate estuaries: model and field observations. Estuarine, Coastal and Shelf Science, 56(2), 305-311.

Tackx, M., De Pauw, N., Van Mieghem, R., Azémar, F., Hannouti, A., Van Damme, S., Fiers, S., Daro, N. & Meire, P. (2004). Zooplankton in the Schelde estuary, Belgium and the Netherlands. Spatial and temporal patterns. *Journal of Plankton Research 26*, 133-141.

Turner, J. T., Ianora, A., Miralto, A., Laabir, M., & Esposito, F. (2001). Decoupling of copepod grazing rates, fecundity and egg-hatching success on mixed and alternating diatom and dinoflagellate diets. Marine Ecology Progress Series, 220(187), e199.

V, W --

Van Damme S., Struyf, E., Maris, T., Ysebaert, T., Dehairs, F., Tackx, M. & Meire, P. (2005). Spatial and temporal patters of water quality along the estuarine salinity gradient of the Scheldt estuary (Belgium and The Netherlands): results of an integrated monitoring approach. *Hydrobiologia 540*, 29 -45.

Van den Meersche, K., Van Rijswijk, P., Soetaert, K. & Middelburg, J.J. (2009). Autochthonous and allochthonous contribution to mesozooplankton diet in a tidal river and estuary: Integrating carbon isotope and fatty acid constraints. Limnology and Oceanography 54(1), 62-74.

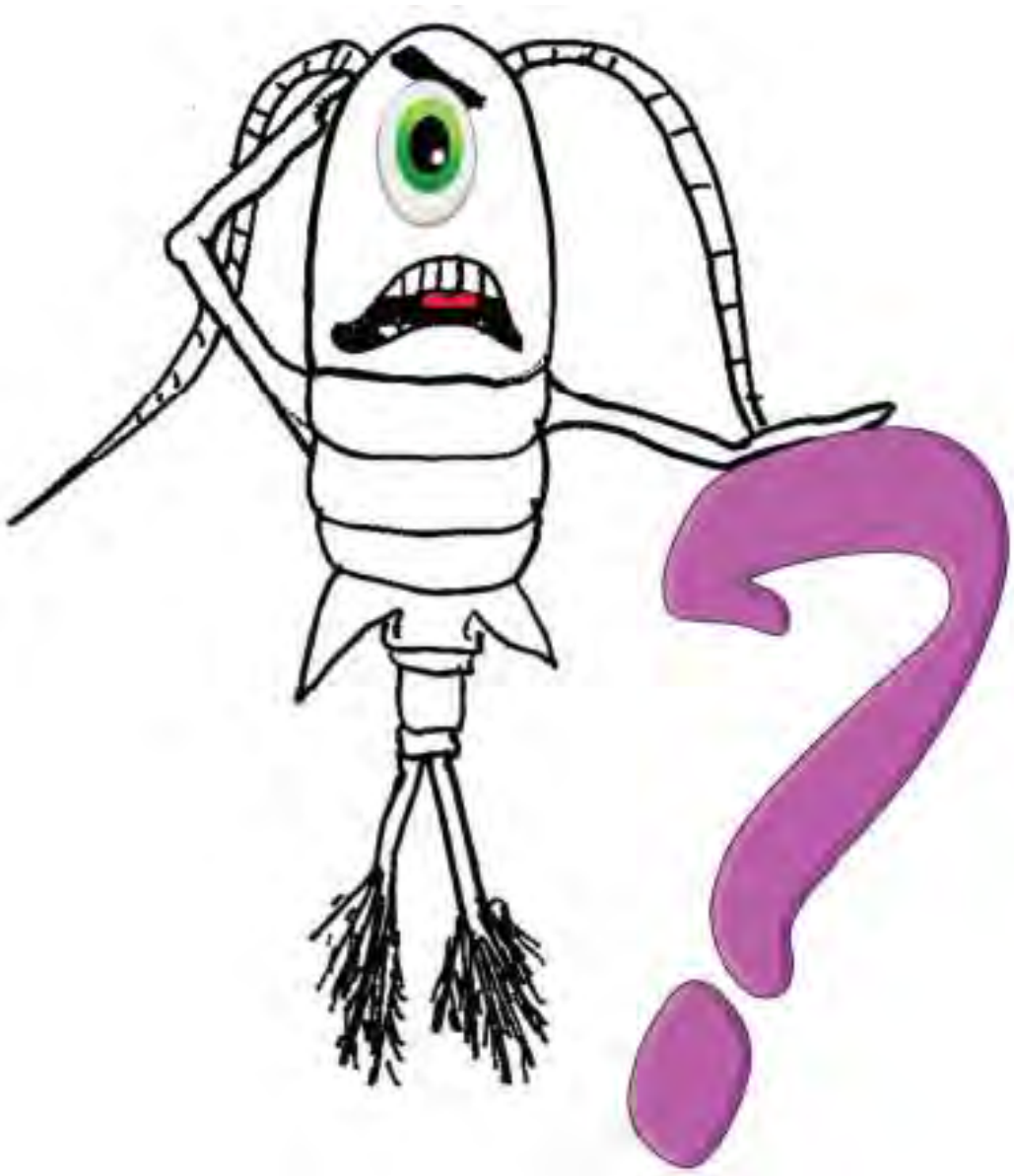
Wada, E., Mizutani, H. & Minagawa, M. (1991). The use of stable isotopes for food web analysis. Critical Reviews in Food Science & Nutrition, 30(4), 361-371.

Winkler, G., & Greve, W. (2004). Trophodynamics of two interacting species of estuarine mysids, *Praunus flexuosus* and *Neomysis integer*, and their predation on the calanoid copepod *Eurytemora affinis*. Journal of experimental marine Biology and Ecology, 308(1), 127-146.

Wright, S. W. (1991). Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Mar. Ecol. Prog. Ser., 77, 183-196.

V. CHAPITRE 5:

Synthèse, discussion générale et perspectives



V.1 Synthèse

Les communautés zooplanctoniques de l'estuaire de l'Escaut en eau saumâtre ont fait l'objet de plus d'attention que celles de la zone d'eau douce (De Pauw, 1973 ; De Pauw, 1975 ; Soetaert et Van Rijswijk, 1993 ; Tackx et al., 1995 ; Irigoien et al., 2000 ; Tackx et al., 2004 ; Tackx et al., 2005 ; Azémar et al., 2007, Mialet et al., 2010). Les travaux de thèse d'Azémar (2007) et de Mialet (2010) soulignent l'importance de la zone d'eau douce, qui héberge des communautés zooplanctoniques plus abondantes et plus diversifiées que la zone d'eau saumâtre.

La restauration de l'Escaut a conduit à des modifications de la distribution spatiale et temporelle du zooplancton. Mialet et al. (2010 ; 2011) rapportent l'installation de la population du copépode calanoïde *Eurytemora affinis*, dominant jusqu'en 2007 en eau saumâtre, dans le tronçon d'eau douce de l'Escaut, et une forte diminution des abondances des copépodes cyclopoïdes dans ce même tronçon. Le premier objectif de ces travaux de thèse était de vérifier la stabilité de cette 'nouvelle' communauté zooplanctonique en étudiant la communauté méso-zooplanctonique en relation avec les facteurs environnementaux après ajout de données complémentaires concernat les années 2010-2012. Ce travail a aussi permis de mettre en évidence la structure spatio-temporelle du méso-zooplancton dans la zone d'eau douce. L'abondance des cladocères a révélé une stabilisation avec un pic d'abondance en 2006 à Dendermonde et Uitbergen. Les copépodes calanoïdes, dominés par *E. affinis*, se sont introduits dans la zone d'eau douce tout d'abord au printemps 2001, pour connaitre un accroissement spectaculaire en 2007. Bien que moins marqué à Melle, l'augmentation de l'abondance d'*E. affinis* a été croissante depuis 2007 (cf. chapitre 1). En effet, l'espèce est aujourd'hui plus abondante dans la zone d'eau douce que dans la zone d'eau saumâtre. Parallèlement, les cyclopoïdes ont continué à diminuer en abondance.

Vu sa dominance, nos recherches sur la nutrition du zooplancton de l'Escaut se sont concentré sur *E. affinis*. Les deux méthodes utilisées ; des expériences d'incubation utilisant de l'eau naturelle de l'Escaut et des adultes + CV d'*E. affinis*, et des analyses pigmentaires des contenus stomacaux donnent des taux d'ingestion comparables sur le phytoplancton total (0.55 ± 0.51 ng ind.$^{-1}$ jour^{-1}). Le dosage des pigments par HPLC a été appliqué aussi bien à la quantification de la concentration du phytoplancton dans les expériences d'incubation qu'aux analyses des contenus

pigmentaires stomacaux. Ceci a permis, grace aux pigments biomarquers, de détecter la sélectivté de broutage sélectif sur des groupes d'algues, mais les résultats des deux méthodes diffèrent. Les incubations relèvent une sélectivité pour les diatomées, tandis que les contenus stomacaux indiquent un broutage sur les cryptophycées.

L'impact du broutage de la population d'*E. affinis* sur la communaté phytoplanctonique semble peu importants : 4.5% jour^{-1}. Des expériences de broutage de la communauté microzooplanctonique ont été entamées, mais les résultats sont à ce jour difficilement exploitable.

V.2 Discussion générale et perspectives

V.2.1 Morphométrie et trade-off

Aujourd'hui la biodiversité est une préoccupation partagée notamment entre scientifiques et gestionnaires. Il est donc important de comprendre l'influence des facteurs environnementaux sur la distribution des espèces. Dans le tronçon d'eau saumâtre de l'Escaut, *E. affinis* atteignait son maximum d'abondance au printemps (avril-mai) tandis que les cyclopoïdes, dominant en eau douce, montraient leur pic d'abondance en été. Il y avait donc une ségrégation spatiale et temporelle entre les deux taxons. Nous avons pu constater que le pic d'abondance d'*E. affinis* en eau douce se décale dans le temps et arrive de plus en plus tard (juin). Ce décalage pourrait entrainer une « compétition » entre ces deux taxa. De plus, comme il a été souligné dans cette étude (chapitre I), la concentration en oxygène est un facteur environnemental prépondérant dans la structure des communautés méso-zooplanctoniques de l'Escaut. Afin d'évaluer plus précisément la tolérance d'*E. affinis* à l'hypoxie, des expériences en laboratoire ont été réalisées (chapitre II). Nos résultats ont montré une nette diminution des taux de mortalité chez les calanoïdes avec l'augmentation de la concentration en oxygène, mais pas de relation entre les taux de mortalité des cyclopoïdes et la concentration en oxygène.

Etant donnée la forte relation de la distribution spatiale d'*E. affinis* avec les variables environnementales, son identification aisée, sa position clé dans le réseau trophique estuarien (Fockedey et al., 1999), et sa dominance des communautés de zooplancton dans la plupart des estuaires de l'hémisphère nord (Mialet et al., 2010, Chapitre 1), l'espèce est une bonne candidate au rôle d'indicateur de la qualité

écologique des estuaires (Ben Radhia-Souissi, 2010). Certaines études, menées sur la Gironde, ont montré que les variations morphométriques seraient une réponse à un compromis entre l'énergie dépensée dans l'osmorégulation et celle dépensée dans le développement et la reproduction (Feurtet et al., 1987). Il est alors possible de penser que le repositionnement d'*E. affinis* vers les eaux douces, son optimum écologique dans d'autres estuaires et probablement initial, lui permettent d'optimiser son développement et sa reproduction grâce à un effort d'osmorégulation moindre, autorisant l'allocation d'une plus grande quantité d'énergie vers les compartiments reproductif et de croissance (Cody, 1966). Dans ce contexte, Lambert (2012) a réalisé une étude afin de vérifier si les caractéristiques morphométriques (taille et poids) d'*E. affinis* dénotent des différences d'état physiologique entre les copépodes des différentes zones halines de l'estuaire, dans le but de mieux comprendre les raisons du repositionnement spatial de l'espèce. De plus, cette étude permet de vérifier la pertinence de l'utilisation des caractères morphologiques d'*E. affinis* pour en faire un indicateur écologique. Cette étude a montré à la fois une augmentation de la taille de la population chez les adultes + CV, mais aussi du poids individuel dans l'eau saumâtre entre 2002-2010. *E. affinis* n'étant présent en eau douce que depuis 2007, seulement deux années ont été analysées.

L'étude de traits morphométriques peut permettre de voir si l'amélioration de la qualité de l'eau dans l'estuaire peut être mise en parallèle avec une amélioration de la condition d'*E. affinis*. Afin d'estimer l'évolution physique de la population en eau douce et ainsi essayer d'analyser l''état de santé' (via les traits morphométriques) de cette population, la continuité des travaux de Lambert (2012), serait à envisager. Les histoires de vie évoluent en réponse à des pressions sélectives de l'environnement, mais sont limitées par la variance génétique et l'histoire phylogénétique (Partridge et Harvey 1988; Ben Radhia-Souissi, 2010). Toutes les caractéristiques du cycle de vie qui déterminent le succès de reproduction ne peuvent être maximisées simultanément ; tout investissement dans un seul trait de l'histoire de vie peut se faire au détriment des autres (Stearns 1989, 1992). C'est pourquoi il serait intéressant de coupler des analyses de traits morphométriques avec des analyses sur les trade-off entre le nombre et la taille des œufs chez ce copépode.

V.2.2 Tolérance des taxons

Lors de nos expériences de tolérance envers des basses concentrations en O_2, seul *E. affinis* était identifié à l'espèce, alors que pour les cyclopoïdes le mélange naturel d'espèces a été utilisé. Sachant que les tolérances pour la concentration en oxygène varient selon l'espèce, il serait intéressant de refaire des expériences en identifiant les individus jusqu'à l'espèce. Le changement de la communauté zooplanctonique a été très abrupt : entre 2006 et 2008, *E. affinis* est devenu très dominant et les cyclopoïdes ont quasiment disparu. Ceci a posé des problèmes dans les tests. Afin d'analyser en même temps les tolérances d'*E. affinis* et des cyclopoïdes, il faudrait soit une veille pendant la période printemps-été afin d'obtenir une communauté suffisamment mixte (cyclopoïdes – *E. affinis*), soit maintenir en culture des cyclopoïdes.

V.2.3 Allelopathie ?

Nous n'avons pas pu identifier le(s) facteurs environnementaux responsable(s) de la diminution des abondances des copépodes cyclopoïdes en parallèle du développement d'*E. affinis* en eau douce de l'Escaut. Les rares études qui discutent l'abondance d'autres taxons zooplanctoniques dans des milieux où *E. affinis* est présent, suggèrent que cette espèce est souvent le taxon dominant. C'est le cas, par exemple, dans le tronçon amont de la rivière Ohio (USA) (Counahan et al., 2005), dans des lacs au nord de la Hongrie (Vad et al., 2012) et dans la partie fluviale de l'estuaire de la Gironde (France) (Dininaud, 2015). Une possibilité non-étudiée est l'existence d'effets allélopathiques. Folt & Goldman (1981) ont en effet démontré que le copépode *Epischura nevadensis* du lac Tahoe (USA) excrète des substances chimiques qui réduisent l'activité de filtration de son compétiteur *Diaptomus tyrelli*. Bien que l'allélopathie soit essentiellement considérée comme associée à des plantes terrestres et des macrophytes, elle a également été démontrée chez des coraux, des éponges et des ascidies (Jackson & Buss, 1975 ; Maida et al., 2001 ; Singh & Thakur, 2016). Il est curieux que l'étude de Folt & Goldman (1981), n'ait pas eu de suite par des études sur l'allélopahie dans le monde zooplanctonique, même si le phénomène est bien connu pour des espèces phytoplanctonique (e.g. Van Wichelen et al., 2012 ;

Allen et al., 2014, Leflaive at al., 2014 ; Poulson –Ellenstad et al., 2014 ; Qui et al., 2014).

Les individus d'*E. affinis* dans l'Escaut sont souvent infectés d'épibiontes (Lambert, 2012). Une étude préliminaire a permis d'identifier des ciliés et des champignons comme étant des épibiontes *d'E. affinis* dans l'Escaut. Des études, ont aussi montré la présence d'algues épibiontes sur le zooplankton (e.g. Sirnadel et al., 1997). On pourrait donc se demander si cerrtaines de ces association ou intégrations de composés allélopathiques dans les tissus et / ou carapaces des copépodes aurait pu se produire et ainsi lui conférer des capacités allélopathiques ?

V.2.4 Rôle trophique d'*E. affinis*

Les expériences de broutage ont montré une sélectivité d'*E. affinis* adultes et CV pour les diatomées, mais un faible impact sur le stock de phytoplancton (4,5 % du stock au mois d'avril 2013-2014). Les rations journalières en termes de carbone calculé à partir de nos résultats sont conformes aux valeurs de la littérature pour les copépodes calanoïdes. Mais la fourchette de ces valeurs est très large et ne permet pas de tirer des conclusions claires sur la suffisance de la nutrition herbivore pour ce copépode. Malgré de nombreuses indications révélant qu'*E. affinis* dans l'Escaut est essentiellement herbivore, il nous semble indiqué de vérifier dans quelle mesure cette nourriture pourrait être complétée par d'autres ressources. Notamment considérant le problème des productions de chlorophycées dans les microcosmes expérimentaux, il serait intéressant de vérifier si *E. affinis* n'exerce pas également une activité carnivore dans les eaux douces de l'Escaut.

V.2.5 Comparaison inter-estuaires :

Le complexe d'espèces *E. affinis* est présent dans la plupart des estuaires tempérés Européens et Nord-Américains. Winkler et al. (2011) ont montré que les *E. affinis* présents dans l'Escaut et la Seine appartiennent au même clade. *E. affinis* a montré une réactivité à l'amélioration de la qualité de l'eau dans l'Escaut (Appeltans et al., 2003 ; Mialet et al., 2010; 2011). De plus, l'espèce *E. affinis* figure parmi les quelques modèles biologiques mis en avant dans le cadre de plusieurs programmes de

recherche menés dans l'estuaire de la Seine (e.g. Mouny, 1998 ; Souissi et al., 2007 ; Devreker et al., 2009 ; Souissi et al., 2010).

Les estuaires de L'Escaut et de la Seine sont comparables sur le plan climatique, hydrodynamique et concernant leurs problématiques multi-usages (activités portuaires importantes, aménagements) (Dauvin & Destroy, 2006 ; GIP Seine-aval, 2010). Pourtant, des études récentes menées dans le cadre du projet 'ZooSeine' ont montré que l'abondance et la diversité du zooplancton (copépodes, cladocères, rotifères) sont plus élevées dans l'Escaut que dans la Seine, surtout sur le tronçon d'eau douce (Azémar et al., 2011). Les quantifications des abondances du zooplancton dans la Seine ont été réalisées sur la base d'échantillonnages à plusieurs profondeurs (Devreker et al., 2011, Schmitt et al., 2011), tandis que dans l'Escaut, les échantillonnages pour le zooplancton sont faits en surface. Ce facteur devra être pris en compte lors des comparaisons inter-estuaires, car les abondances peuvent être fortement affectées par la profondeur (Mouny & Dauvin, 1996 ; Devreker et al., 2008 ; Schmitt et al., 2011).

L'abondance et la diversité du zooplancton sont d'une part déterminées par le potentiel de production offert par le milieu : la disponibilité de ressources alimentaires (MES assimilable, phytoplancton, proies animales) et les conditions physico-chimiques du milieu (température, hydrodynamisme, qualité de l'eau). D'autre part, la production de zooplancton réalisée entrera de façon plus ou moins importante dans le réseau trophique, par la prédation exercée par l'hyperbenthos et les poissons, ou par la décomposition des organismes morts par d'autres causes (qualité de l'eau insuffisante, par exemple). Le stock observé in situ (= abondance numérique ou exprimé en biomasse) est le résultant de ces flux.

Une comparaison inter-estuaires des stocks zooplanctoniques observés permettrait de tester certaines hypothèses qui se situent dans le contexte du contrôle des stocks/productions par le 'bottom-up/top down' (McQueen et al., 1989). Une étude préliminaire de la concentration et composition de la matière en suspension (MES) montre que la matière organique dans l'Escaut contient plus de phytoplancton vivant que celle de la Seine. Au vu des connaissances sur la sélectivité des copépodes estuariens envers le phytoplancton (Gasparini et al., 1999 ; Tackx et al., 2003; Azémar, 2007 ; Chapitre III), cette comparaison permettrait de tester l'hypothèse

selon laquelle la composition de la matière en suspension est un facteur expliquant le plus grand 'succès' du zooplancton dans l'Escaut que dans la Seine.

Cette hypothèse pourrait être testée par des mesures de production du zooplancton dans le milieu naturel (en excluant les prédateurs), en comparant ce 'potentiel de production' dans la Seine et dans l'Escaut. En partant du cas le plus probable que la production est plus faible dans la Seine que dans l'Escaut, il s'agira essentiellement de détecter si l'ajout de phytoplancton augmente la production du zooplancton dans la Seine. Dans ce cas, la disponibilité de phytoplancton est le facteur limitant la production du zooplancton dans la Seine. Dans le cas contraire, la qualité de l'eau pourrait être responsable d'une faible production du zooplancton dans la Seine.

V.2.6 Le méso-zooplancton dans les estuaires mais aussi dans d'autres milieux :

Le zooplancton peut aussi être observé dans d'autres milieux que les zones pélagiques des estuaires, des océans et des mers. Dans certains milieux, comme dans les lacs, le zooplancton peut être abondant ou encore se 'cacher' auprès des végétations macrophytes et/ou litières. Cela est traditionnemllement considéré comme une action de refuge contre la prédation, mais la possibilité d'un aspet trophique n'a presque pas été considérée.

Jusqu'à présent les études sur le compartiment mesofaunistique se sont essentiellement concentrées sur l'étude des assemblages mésoplanctoniques des zones pélagiques que ce soit en milieu lacustre, estuarien ou marin. Ces études se sont focalisées sur la distribution et la structuration des assemblages et le rôle trophique du mésozooplancton. Le couplage des habitats benthiques et littoraux ainsi que le rôle fonctionnel des communautés mésobenthiques ont été largement sous-explorés. Pourtant, l'étude du fonctionnement des communautés benthiques est particulièrement importante pour comprendre les mécanismes de réponses des communautés pélagiques dans la mesure où ces deux types d'habitat sont interconnectés et structurés par des échanges de matière et d'énergie, primordiaux pour le fonctionnement biogéochimique des écosystèmes. Par exemple, les habitats pélagiques constituent une source de matière organique pour les habitats benthiques via la sédimentation du plancton (Covich et al., 1999) et de proies pour les invertébrés benthiques (e.g. MacIsaac et al., 1992). En interceptant la lumière et les nutriments,

les réseaux trophiques pélagiques ont une incidence sur les processus benthiques (Sand-Jensen and Borum, 1991; Vadeboncoeur et al., 2002). La zone benthique fournit également des proies pour les prédateurs pélagiques comme les poissons (e.g. Stein et al., 1995) et des habitats refuges (e.g. Cáceres, 1997). Elle est le siège de nombreux processus biologiques permettant la remobilisation de nutriments via les processus métaboliques des organismes benthiques et via la reminéralisation de la matière organique (Vanni, 1996). Le rôle de la meiofaune dans la reminéralisation de la matière organique a été suggéré à de nombreuses reprises. De nombreux travaux en milieux marins et en zones pélagiques d'eau douce soulignent le lien trophique entre la mésofaune et les communautés microbiennes associées à la matière organique et les macroinvertébrés détritivores. (e.g. Kemp 1990; Golladay & Hax 1995; Robertson, Lancaster & Hildrew 1995; Freckman et al. 1997; Hakenkamp & Morin 2000; Schmid-Araya & Schmid 2000; Swan & Palmer 2000). Malgré cette évidence et de nombreux appels à développer des études sur le rôle fonctionnel de la mésofaune dans le réseau trophique hétérotrophe, très peu d'études ont été réalisées sur le sujet. Dans le cadre de ma thèse et en collaboration avec les membres du laboratoire qui travaillent sur les réseaux hétérotrophes, j'ai réalisé une expérimentation qui avait pour objectif d'identifier le rôle de la mésofaune dans la dégradation de la matière organique (Annexe 1). Nous supposons que la mésofaune influence la structure des assemblages microbiens associés à la matière organique par son activité de broutage et par conséquent leur contribution aux processus de dégradation de la matière organique. En interaction avec le compartiment macrobenthique, nous supposons que l'introduction de la mésofaune dans le réseau trophique hétérotrophe influe sur des interactions trophiques entre les organismes. Ceci pourrait être lié à une augmentation de l'efficacité des processus de dégradation de la matière organique via des effets additifs ou de complémentarité (ex : facilitation ou partitionnement de la ressource) entre compartiments, ou encore à une diminution des taux de dégradation de la matière organique via des interactions négatives entre compartiments, telles que la compétition ou la prédation. Notre étude confirme que la mésofaune peut jouer un rôle important dans le réseau hétérotrophe aquatique, notamment via son interaction avec les communautés microbiennes et de macroinvertébrés déchiqueteurs.

V.2.7 Références bibliographiques

A, B --

Appeltans, W., Hannouti, A., van Damme, S., Soetaert, K., Vanthomme, R. & Tackx, M. (2003). Zooplankton in the Schelde estuary (Belgium/The Netherlands). The distribution of *Eurytemora affinis*: effect of oxygen? Journal of Plankton Research, 25(11), 1441-1445.

Azémar, F. (2007). L'écologie du zooplancton de l'estuaire de l'Escaut (Belgique). (Thèse de doctorat, Université Paul Sabatier Toulouse III).

Azémar F., Julien, F., Druilhe, J.L., Sossou, C., Souissi, S. & Tackx, M. (2011). The zooplankton of the Seine estuary Rapport projet Zooseine. 20 pp.

Ben Radhia-Souissi, A. (2010) Etude de la plasticité et de la morphologie d'un copépode estuarien : comparaison intercontinentale. (Thèse de doctorat, Université de Lille 1).

C--

Cáceres, C. E. (1997). Dormancy in invertebrates. Invertebrate biology, 371-383.

Cody, M. L. (1966). A general theory of clutch size. Evolution, 174-184.

Covich, A. P., Palmer, M. A., & Crowl, T. A. (1999). The role of benthic invertebrate species in freshwater ecosystems: zoobenthic species influence energy flows and nutrient cycling. BioScience, 49(2), 119-127.

Counahan, D. F., Carline, R. F., & Reid, J. W. (2005). The occurrence of the calanoid copepod *Eurytemora affinis* (Poppe) in the upper Ohio River. Northeastern Naturalist, 12(4), 541-545.

D --

Dauvin, J. C., Desroy, N., Janson, A. L., Vallet, C., & Duhamel, S. (2006). Recent changes in estuarine benthic and suprabenthic communities resulting from the development of harbour infrastructure. Marine Pollution Bulletin, 53(1), 80-90.

De Pauw, N. (1973). On the distribution of *Eurytemora affinis* (Poppe)(Copepoda) in the Western Scheldt estuary. Verhandlungen. Internationale Vereinigung für theoretische und angewandte Limnologie.

De Pauw, N. (1975). Bijdrage tot de kennis van milieu en plankton in het Westerschelde estuarium. PhD, thesis University of Ghent (Belgium). I, Dutch.

Devreker, D., Souissi, S., & Seuront, L. (2004). Development and mortality of the first naupliar stages of *Eurytemora affinis* (Copepoda, Calanoida) under different conditions of salinity and temperature. Journal of Experimental Marine Biology and Ecology, 303(1), 31-46.

Devreker, D., Souissi, S., Forget-Leray, J., & Leboulenger, F. (2007). Effects of salinity and temperature on the post-embryonic development of *Eurytemora affinis* (Copepoda; Calanoida) from the Seine estuary: a laboratory study. Journal of Plankton Research, 29(suppl 1), i117-i133.

Devreker, D., Souissi, S., Molinero, J. C., & Nkubito, F. (2008). Trade-offs of the copepod *Eurytemora affinis* in mega-tidal estuaries: insights from high

frequency sampling in the Seine estuary. Journal of Plankton Research, 30(12), 1329-1342.

Devreker, D., Souissi, S., Winkler, G., Forget-Leray, J. & Leboulenger, F. (2009). Effects of salinity, temperature and individual variability on the reproduction of *Eurytemora affinis* (Copepoda; Calanoida) from the Seine estuary: a laboratory study. Journal of Experimental Marine Biology and Ecology, 368(2), 113-123.

Devreker, D., Souissi, S., Molinero, J. C., Beyrend-Dur, D., Gomez, F., & Forget-Leray, J. (2010). Tidal and annual variability of the population structure of *Eurytemora affinis* in the middle part of the Seine Estuary during 2005. Estuarine, Coastal and Shelf Science, 89(4), 245-255.

F --

Feurtet, A., & Castel, J. (1987). Biologie du copépode *Eurytemora affinis hirundoides* dans la Gironde: données morphométriques. In Aspects récents de la biologie des crustacés. Concarneau (France)

Fockedey, N., & Mees, J. (1999). Feeding of the hyperbenthic mysid *Neomysis integer* in the maximum turbidity zone of the Elbe, Westerschelde and Gironde estuaries. Journal of Marine Systems, 22(2), 207-228.

Folt, C., & Goldman, C. R. (1981). Allelopathy between zooplankton: a mechanism for interference competition. Science, 213(4512), 1133-1135.

Freckman, D. W., Blackburn, T. H., Brussaard, L., Hutchings, P., Palmer, M. A., & Snelgrove, P. V. (1997). Linking biodiversity and ecosystem functioning of soils and sediments. Ambio, 556-562.

G, H --

Gasparini, S., Castel, J., & Irigoien, X. (1999). Impact of suspended particulate matter on egg production of the estuarine copepod, *Eurytemora affinis*. Journal of Marine Systems, 22(2), 195-205.

GIP Seine-aval, 2010.

Golladay, S. W., & Hax, C. L. (1995). Effects of an engineered flow disturbance on meiofauna in a north Texas prairie stream. Journal of the North American Benthological Society, 404-413.

Hakenkamp, C. C., & Morin, A. (2000). The importance of meiofauna to lotic ecosystem functioning. Freshwater biology, 44(1), 165-175.

Hu, S., Guo, Z., Li, T., Carpenter, E. J., Liu, S., & Lin, S. (2014). Detecting in situ copepod diet diversity using molecular technique: development of a Copepod/Symbiotic Ciliate-Excluding Eukaryote-Inclusive PCR Protocol. PloS one, 9(7), e103528.

I, J --

Irigoien, X., Head, R. N., Harris, R. P., Cummings, D., Harbour, D., & Meyer-Harms, B. (2000). Feeding selectivity and egg production of *Calanus helgolandicus* in the English Channel. Limnology and Oceanography, 45(1), 44-54.

Jackson, J. B. C., & Buss, L. (1975). Alleopathy and spatial competition among coral reef invertebrates. Proceedings of the National Academy of Sciences, 72(12), 5160-5163.

K, L --

Kemp, W. M., Sampou, P., Caffrey, J., Mayer, M., Henriksen, K., & Boynton, W. R. (1990). Ammonium recycling versus denitrification in Chesapeake Bay sediments. Limnology and Oceanography, 35(7), 1545-1563.

Lambert, C. (2012). Etude des caractéristiques morphométriques d'*Eurytemora affinis* (Poppe, 1880, copepodes, calanoïdes) comme indicatrices de qualité du milieu. (Rapport Stage M1 Ecologie Université Paul Sabatier Toulouse III).

Leflaive, J., & Ten-Hage, L. (2009). Chemical interactions in diatoms: role of polyunsaturated aldehydes and precursors. New Phytologist, 184(4), 794-805.

M --

MacIsaac, H. J., Sprules, G., Johannson, O. E., & Leach, J. H. (1992). Filtering impacts of larval and sessile zebra mussels (*Dreissena polymorpha*) in western Lake Erie. Oecologia, 92(1), 30-39.

Maida, M., Sammarco, P. W., & Coll, J. C. (2001). Effects of soft corals on scleractinian coral recruitment. II: Allelopathy, spat survivorship and reef community structure. Marine Ecology, 22(4), 397-414.

McQueen, D. J., Johannes, M. R., Post, J. R., Stewart, T. J., & Lean, D. R. (1989). Bottom-up and top-down impacts on freshwater pelagic community structure. Ecological Monographs, 59(3), 289-309.

Mialet, B. (2010). Réponse du zooplancton à la restauration de l'estuaire de l'Escaut et test d'un modèle de sélectivité trophique (Thèse de doctorat. Université Paul Sabatier Toulouse III).

Mialet, B., Azémar, F., Maris, T., Sossou, C., Ruiz, P., Lionard, M., Van Damme, S., Lecerf, A., Muylaert, K., Toumi, N. & Tackx, M. (2010). Spatial spring distribution of the copepod *Eurytemora affinis* (Copepoda, Calanoida) in a restoring estuary, the Scheldt (Belgium), Estuarine, Coastal and Shelf Science, 88, 116-124.

Mialet, B., Gouzou, J., Azémar, F., Maris, T., Sossou, C., Toumi, N. & Tackx, M. (2011). Response of zooplankton to improving water quality in the Scheldt estuary (Belgium). Estuarine, Coastal and Shelf Science, 93(1), 47-57.

Mouny, P., & Dauvin, J. C. (1996). Les communautés mesozooplanctoniques et suprabenthiques de l'estuaire de la Seine. *Rapport d'activité du Programme ScientifiqueSeine-Aval'. Rapport*, 62-98.

Mouny, P. (1998). Structure spatio-temporelle du zooplancton et du suprabenthos de l'estuaire de la Seine. Dynamique et rôle des principales espèces dans la chaîne trophique pélagique SYNTHESE (Doctoral dissertation, Muséum National d'Histoire Naturelle).

P, Q --

Partridge, L., & Harvey, P. H. (1988). The ecological context of life history evolution. Science, 241(4872), 1449-1455.

Qiu, X., Shimasaki, Y., Yoshida, Y., Matsubara, T., Yamasaki, Y., Kawaguchi, M. & Kuno, K. (2014). Allelopathic Effects of *Skeletonema* spp. May Influence Interspecific Competition and Bloom Formation of Co–occurring Harmful Flagellates. Journal of the Faculty of Agriculture, Kyushu University, 59(2), 373-382.

R, S --

Robertson, A. L., Lancaster, J., & Hildrew, A. G. (1995). Stream hydraulics and the distribution of microcrustacea: a role for refugia? Freshwater Biology, 33(3), 469-484.

Sand-Jensen, K., & Borum, J. (1991). Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquatic Botany, 41(1), 137-175.

Schmid-Araya, J. M., & Schmid, P. E. (2000). Trophic relationships: integrating meiofauna into a realistic benthic food web. Freshwater Biology, 44(1), 149-163.

Schmitt, F. G., Devreker, D., Dur, G., & Souissi, S. (2011). Direct evidence of tidally oriented behavior of the copepod *Eurytemora affinis* in the Seine estuary. Ecological research, 26(4), 773-780.

Singh, A., & Thakur, N. L. (2016). Significance of investigating allelopathic interactions of marine organisms in the discovery and development of cytotoxic compounds. Chemico-biological interactions, 243, 135-147.

Soetaert, K. & Van Rijswijk, P. (1993). Spatial and temporal patterns of the zooplankton in the Westerschelde estuary. Marine ecology progress series. 97(1), 47-59.

Souissi, S., Forget-Leray, J., Budzinski, H., Dauvin, J. C., Schmitt, F. G., Hwang, J. S., ... & Devreker, D. (2007). Synthèse des travaux réalisés sur le modèle biologique *Eurytemora affinis* dans Seine-Aval 3.

Souissi, A., Souissi, S., Devreker, D., & Hwang, J. S. (2010). Occurence of intersexuality in a laboratory culture of the copepod *Eurytemora affinis* from the Seine estuary (France). Marine biology, 157(4), 851-861.

Stearns, S. C. (1989). Trade-offs in life-history evolution. Functional ecology, 3(3), 259-268.

Stearns, S. C. (1992). The evolution of life histories (Vol. 249). Oxford: Oxford University Press.

Stein, R. A., DeVries, D. R., & Dettmers, J. M. (1995). Food-web regulation by a planktivore: exploring the generality of the trophic cascade hypothesis. Canadian Journal of Fisheries and Aquatic Sciences, 52(11), 2518-2526.

Stirnadel, H. A., & Ebert, D. (1997). Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. Journal of Animal Ecology, 212-222.

Swan, C. M., & Palmer, M. A. (2000). What drives small-scale spatial patterns in lotic meiofauna communities? Freshwater Biology, 44(1), 109-121.

T--

Tackx, M., Irigoien, X., Daro, N., Castel, J., Zhu, L., Zhang, X., & Nijs, J. (1995). Copepod feeding in the Westerschelde and the Gironde. Hydrobiologia, 311(1), 71-83.

Tackx, M. L. M., Herman, P. J. M., Gasparini, S., Irigoien, X., Billiones, R., & Daro, M. H. (2003). Selective feeding of *Eurytemora affinis* (Copepoda, Calanoida) in temperate estuaries: model and field observations. Estuarine, Coastal and Shelf Science, 56(2), 305-311.

Tackx, M. L., De Pauw, N., Van Mieghem, R., Azémar, F., Hannouti, A., Van Damme, S. and Meire, P. (2004). Zooplankton in the Schelde estuary, Belgium and The Netherlands. Spatial and temporal patterns. Journal of Plankton Research, 26(2), 133-141.

Tackx, M., Azémar, F., Boulêtreau, S., De Pauw, N., Bakker, K., Sautour, B., Gasparini, S., Soetaert, K., Van Damme, S., & Meire, P. (2005). Zooplankton in the Schelde estuary, Belgium and the Netherlands: long-term trends in spring populations. Hydrobiologia, 540(1-3), 275-278.

Tackx, M., F. Azémar, S. Boulêtreau, N. D. Pauw, K. Bakker, B. Sautour, S. Gasparini, K. Soetaert, S. V. Damme, & P. Meire (2005). Zooplankton in the Schelde estuary, Belgium and the Netherlands: long-term trends in spring populations. Hydrobiologia 540: 275–278.

V, W--

Vad, C. F., Horvath, Z., Kiss, K. T., & Acs, E. (2012). Microcrustacean (Cladocera, Copepoda) communities in artificial lakes in the region of the North Hungarian Mountains, with special reference to the adventive species. Acta Zoologica Academiae Scientiarum Hungaricae, 58(1), 47-61.

Vadeboncoeur, Y., Vander Zanden, M. J., & Lodge, D. M. (2002). Putting the Lake Back Together: Reintegrating Benthic Pathways into Lake Food Web Models Lake ecologists tend to focus their research on pelagic energy pathways, but, from algae to fish, benthic organisms form an integral part of lake food webs. Bioscience, 52(1), 44-54.

Vanni, M. J. (1996). Nutrient transport and recycling by consumers in lake food webs: implications for algal communities. In Food Webs (pp. 81-95). Springer US.

Van Wichelen, J., van Gremberghe, I., Vanormelingen, P., & Vyverman, W. (2012). The importance of morphological versus chemical defences for the bloom-forming cyanobacterium *Microcystis* against amoebae grazing. Aquatic Ecology, 46(1), 73-84.

Winkler, G., Souissi, S., Poux, C. & Castric, V. (2011). Genetic heterogeneity among *Eurytemora affinis* populations in Western Europe. Marine biology 158, 1841–1856.

VI. ANNEXES

VI.1 Annexe 1

Tableau 8 : Results of each 12 grazing experiments (from I to IX in 2013 and from X to XIII in 2014) with *E. affinis*. Standard deviation for concentration of each pigments (n=3 for 2013; n=6 for 2014) at t0, in control (tcontrol) and treatment (tzoo) microcosms. Standard deviation for ingestion and clearance rates (n=3 for 2013; n=6 for 2014)

Standard deviation t_0 (µg L^{-1})

Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
I	0.179	0.005	0.035	0.010	0.013	0.026	0.018	0.036	0.394	0.146	0.096	0.005
II	0.087	0.008	0.022	0.003	0.013	0.019	0.021	0.009	0.380	0.092	0.052	0.011
III	0.400	0.001	0.081	0.015	0.027	0.012	0.073	0.045	1.063	0.119	0.277	0.007
IV	0.065	0.011	0.012	0.012	0.021	0.019	0.028	0.027	0.260	0.061	0.262	0.006
V	0.024	0.004	0.001	0.004	0.010	0.004	0.010	0.007	0.101	0.034	0.042	0.011
VI	0.098	0.009	0.013	0.001	0.031	0.027	0.028	0.019	0.398	0.034	0.326	0.008
VII	0.098	0.011	0.018	0.003	0.016	0.017	0.007	0.006	0.091	0.042	0.046	0.110
VIII	0.108	0.007	0.024	0.003	0.007	0.011	0.004	0.011	0.088	0.039	0.083	0.028
IX	0.069		0.016	0.007	0.022	0.002	0.010	0.006	0.118	0.035	0.013	0.013
X	0.093	0.005	0.018	0.011	0.022	0.012	0.018	0.007	0.150	0.128	0.118	0.006
XI	0.041	0.002	0.010	0.004	0.008	0.009	0.018	0.010	0.127	0.032	0.076	0.007
XII	0.149	0.008	0.019	0.012	0.050	0.019	0.055	0.016	0.244	0.052	0.057	0.020
XIII	0.263	0.007	0.039	0.026	0.055	0.026	0.029	0.012	0.387	0.127	0.008	0.023

Standard deviation tcontrol (µg L^{-1})

Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
I	0.078	0.012	0.023	0.008	0.015	0.016	0.018	0.025	0.317	0.053	0.314	0.002
II	0.066	0.003	0.017	0.001	0.003	0.008	0.005	0.012	0.128	0.018	0.060	0.001
III	0.083	0.001	0.013	0.010	0.020	0.004	0.026	0.033	0.204	0.017	0.024	0.000
IV	0.045	0.007	0.004	0.010	0.002	0.010	0.013	0.022	0.143	0.030	0.256	0.006
V	0.017	0.004	0.012	0.010	0.004	0.014	0.005	0.002	0.164	0.036	0.303	0.005
VI	1.034	0.006	0.004	0.008	0.020	0.017	0.012	0.016	0.122	0.022	0.131	0.011
VII	0.048	0.002	0.033	0.004	0.015	0.008	0.011	0.011	0.138	0.014	0.174	0.004
VIII	0.037	0.002	0.008	0.008	0.029	0.003	0.019	0.028	0.148	0.026	0.068	0.022
IX	0.073		0.014	0.013	0.005	0.013	0.023	0.005	0.122	0.059		0.005
X	0.070	0.003	0.009	0.009	0.013	0.004	0.009	0.003	0.097	0.028	0.016	0.004
XI	0.106	0.002	0.026	0.012	0.015	0.017	0.017	0.012	0.220	0.050	0.034	0.005
XII	0.198	0.008	0.035	0.020	0.028	0.024	0.044	0.023	0.428	0.059	0.056	0.017
XIII	0.203	0.008	0.020	0.020	0.033	0.015	0.033	0.014	0.366	0.112	0.061	0.011

Standard deviation tzoo (µg L^{-1})

Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
I	0.026	0.096	0.019	0.020	0.032	0.019	0.035	0.032	0.410	0.065	0.478	0.044
II	0.093	0.007	0.010	0.006	0.019	0.019	0.030	0.011	0.249	0.079	0.378	0.016
III	0.086	0.001	0.010	0.009	0.021	0.003	0.009	0.027	0.053	0.174	0.095	0.003
IV	0.090	0.005	0.005	0.003	0.012	0.015	0.013	0.002	0.082	0.042	0.155	0.010
V	0.068	0.007	0.012	0.002	0.030	0.007	0.009	0.013	0.137	0.013	0.024	0.010
VI	0.043	0.007	0.009	0.004	0.028	0.007	0.040	0.002	0.332	3.941	0.305	0.011
VII	0.036	0.005	0.008	0.014	0.017	0.033	0.011	0.016	0.297	0.018	0.197	0.010
VIII	0.061	0.006	0.018	0.007	0.020	0.004	0.013	0.019	0.124	0.087	0.100	0.018
IX	0.011		0.013	0.012	0.015	0.013	0.014		0.017	0.023	0.040	0.016
X	0.113	0.007	0.017	0.020	0.025	0.021	0.012	0.002	0.238	0.064	0.034	0.010
XI	0.062	0.003	0.010	0.006	0.020	0.008	0.021	0.001	0.150	0.079	0.026	0.010
XII	0.099	0.005	0.018	0.010	0.029	0.029	0.026	0.002	0.173	0.066	0.033	0.005
XIII	0.143	0.003	0.025	0.016	0.032	0.015	0.016	0.003	0.079	0.106	0.045	0.018

Standard deviation F (ml ind.$^{-1}$h^{-1})

Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
I	0.059	0.895	0.204	0.316	0.296	0.242	0.414	0.406	0.295	0.263	0.313	0.710
II	0.191	0.211	0.137	0.064	0.176	0.220	0.677	0.347	0.270	0.439	0.215	0.439
III	0.093	0.020	0.045	0.162	0.129	0.022	0.045	0.239	0.022	0.336	0.042	0.061
IV	0.200	0.129	0.067	0.045	0.081	0.159	0.187	0.056	0.066	0.153	0.082	0.233
V	0.122	0.159	0.110	0.035	0.195	0.074	0.140	0.304	0.096	0.063	0.015	0.248
VI	0.051	0.175	0.055	0.056	0.142	0.047	0.410	0.031	0.180	1.798	0.163	0.142
VII	0.078	0.141	0.092	0.208	0.108	0.284	0.264	0.326	0.219	0.079	0.152	0.189
VIII	0.072	0.131	0.103	0.174	0.103	0.032	0.069	0.226	0.070	0.161	0.064	0.258
IX	0.015		0.130	0.166	0.076	0.092	0.181		0.011	0.050	0.138	0.248
X	0.117	0.164	0.123	0.123	0.110	0.124	0.095	0.053	0.130	0.128	0.084	0.100
XI	0.097	0.075	0.112	0.061	0.123	0.058	0.135	0.039	0.120	0.144	0.101	0.134
XII	0.093	0.095	0.136	0.062	0.136	0.224	0.115	0.033	0.079	0.076	0.075	0.045
XIII	0.142	0.065	0.180	0.095	0.130	0.088	0.077	0.053	0.039	0.139	0.110	0.171

Standard deviation I (ng ind.$^{-1}$h^{-1})

Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll *c*	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Zeaxanthin
I	0.037	0.102	0.022	0.013	0.040	0.023	0.034	0.037	0.496	0.110	0.208	0.050
II	0.144	0.009	0.016	0.005	0.027	0.027	0.047	0.014	0.376	0.155	0.171	0.020
III	0.084	0.001	0.011	0.009	0.024	0.004	0.011	0.020	0.056	0.207	0.100	0.004
IV	0.082	0.005	0.005	0.003	0.012	0.016	0.014	0.002	0.084	0.044	0.155	0.009
V	0.063	0.006	0.012	0.002	0.030	0.007	0.008	0.014	0.139	0.014	0.025	0.010
VI	0.033	0.007	0.009	0.004	0.033	0.008	0.050	0.001	0.380	4.417	0.340	0.014
VII	0.033	0.004	0.008	0.015	0.015	0.033	0.008	0.014	0.256	0.016	0.177	0.016
VIII	0.056	0.006	0.019	0.007	0.017	0.004	0.019	0.010	0.157	0.079	0.098	0.016
IX	0.010		0.013	0.011	0.014	0.014	0.012		0.017	0.024	0.028	0.016
X	0.114	0.007	0.019	0.020	0.026	0.021	0.016	0.002	0.238	0.085	0.041	0.010
XI	0.059	0.003	0.011	0.006	0.019	0.008	0.022	0.001	0.141	0.073	0.025	0.009
XII	0.092	0.004	0.016	0.010	0.027	0.030	0.029	0.001	0.156	0.066	0.030	0.006
XIII	0.118	0.003	0.019	0.016	0.029	0.016	0.014	0.003	0.069	0.090	0.042	0.018

VI.2 Annexe 2

Tableau 9 : Results of each 4 grazing experiments with microzooplankton. Standard deviation for concentration of each pigments (n=6) at t_0, in control (tcontrol) and treatment (tzoo) microcosms. Standard deviation for ingestion and clearance rates (n=6)

d deviation t_0 (µg L^{-1})												
Experiment	**Fucoxanthin**	**Violaxanthin**	**Diadinoxanthin**	**Diathoxanthin**	**Lutein**	**Alloxanthin**	**B-carotene**	**Chlorophyll *c***	**Chlorophyll *a***	**Pheophytin *a***	**Pheophorbide *a***	**Zeaxanthin**
X	0.086	0.004	0.014	0.007	0.026	0.005	0.026	0.006	0.272	0.090	0.070	0.014
XI	0.125	0.003	0.021	0.013	0.014	0.013	0.019	0.010	0.179	0.052	0.018	0.004
XII	0.066	0.006	0.010	0.013	0.018	0.009	0.020	0.000	0.166	0.053	0.003	0.012
XIII	0.305	0.002	0.047	0.037	0.056	0.027	0.050	0.037	0.506	0.103	0.126	0.023

leviation $t_{control}$ (µg L^{-1})												
Experiment	**Fucoxanthin**	**Violaxanthin**	**Diadinoxanthin**	**Diathoxanthin**	**Lutein**	**Alloxanthin**	**B-carotene**	**Chlorophyll *c***	**Chlorophyll *a***	**Pheophytin *a***	**Pheophorbide *a***	**Zeaxanthin**
X	0.031	0.003	0.006	0.006	0.012	0.010	0.012	0.006	0.084	0.029	0.045	0.009
XI	0.072	0.003	0.008	0.004	0.006	0.007	0.011	0.006	0.167	0.045	0.014	0.006
XII	0.072	0.010	0.015	0.008	0.011	0.021	0.038	0.020	0.150	0.022	0.036	0.007
XIII	0.211	0.006	0.027	0.027	0.032	0.033	0.024	0.014	0.336	0.070	0.039	0.012

deviation t_{zoo} (µg L^{-1})												
Experiment	**Fucoxanthin**	**Violaxanthin**	**Diadinoxanthin**	**Diathoxanthin**	**Lutein**	**Alloxanthin**	**B-carotene**	**Chlorophyll *c***	**Chlorophyll *a***	**Pheophytin *a***	**Pheophorbide *a***	**Zeaxanthin**
X	0.071	0.003	0.010	0.008	0.014	0.005	0.009	0.003	0.098	0.029	0.015	0.006
XI	0.106	0.002	0.027	0.012	0.015	0.017	0.016	0.007	0.221	0.050	0.008	0.006
XII	0.199	0.008	0.035	0.020	0.029	0.024	0.043	0.006	0.429	0.059	0.065	0.016
XIII	0.203	0.009	0.020	0.020	0.032	0.014	0.033	0.012	0.366	0.113	0.082	0.011

eviation F (ml ind.$^{-1}$h^{-1})												
Experiment	**Fucoxanthin**	**Violaxanthin**	**Diadinoxanthin**	**Diathoxanthin**	**Lutein**	**Alloxanthin**	**B-carotene**	**Chlorophyll *c***	**Chlorophyll *a***	**Pheophytin *a***	**Pheophorbide *a***	**Zeaxanthin**
X	4.205	3.818	4.345	3.448	3.896	2.177	5.953	2.156	3.650	5.966	2.739	3.032
XI	5.512	2.846	14.321	6.157	4.265	11.159	7.099	8.437	6.683	6.192	7.141	4.494
XII	5.933	5.754	6.644	5.913	5.675	8.169	9.679	9.455	6.327	3.959	7.411	8.195
XIII	6.152	9.483	3.742	6.275	5.347	5.709	9.014	7.085	6.052	8.295	7.372	5.458

leviation I (ng ind.$^{-1}$h^{-1})												
Experiment	**Fucoxanthin**	**Violaxanthin**	**Diadinoxanthin**	**Diathoxanthin**	**Lutein**	**Alloxanthin**	**B-carotene**	**Chlorophyll *c***	**Chlorophyll *a***	**Pheophytin *a***	**Pheophorbide *a***	**Zeaxanthin**
X	3.557	0.136	0.486	0.404	0.729	0.247	0.543	0.167	5.409	2.014	0.842	0.278
XI	4.740	#DIV/0!	1.391	0.675	0.713	0.783	1.133	0.514	10.210	2.787	0.598	0.260
XII	9.307	0.318	1.647	0.960	1.492	1.192	2.385	1.131	19.988	3.089	2.649	0.818
XIII	10.265	0.473	1.044	0.790	1.669	0.788	1.713	0.771	18.692	6.366	3.324	0.544

VI.3 Annexe 3

Two freshwater meiofaunal crustaceans affects leaf breakdown by microbial and macroinvertebrate decomposers

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Summary

1. Leaf litter processing is a fundamental ecosystem process in freshwaters performed by a diverse range of decomposers. The meiofauna are an important constituent of aquatic heterotrophic assemblages which can provide a trophic link between plant detritus and associated microbial and macroinvertebrate communities, but their contribution to leaf breakdown remains poorly understood.

2. In this study, we designed experiments to evaluate the potential contribution of freshwater meiofaunal crustaceans (assemblage of one cladoceran and one copepod taxa) to leaf litter breakdown. We considered that interactions with the two main biological compartments involved in this process, microbial decomposers (fungal hyphomycetes) and macroinvertebrate detritivores (a trichopteran and gammarid amphipod) would influence the contribution of meiofaunal assemblages.

3. The contribution of meiofauna was evaluated in laboratory trials with six treatments where fungi were always present (presence/absence of meiofauna crossed with either detritivore presence/absence) and effects determined after 6 and 13 day incubations. We hypothesized that through their feeding activity, meiofauna influences the structure of fungal assemblages and consequently, fungi-mediated leaf litter breakdown. Meiofauna were predicted to change the way energy from organic matter moved through the food web. This was expected to vary according to the trophic relationships with each detritivore, reflecting either positive (e.g. complementarity) or negative (e.g. predation) interactions.

4. The presence of leaf-associated meiofauna enhanced fungi-mediated leaf mass loss by 62% and by trichopteran-driven leaf mass loss by 22% while no significant effect was observed on amphipod-driven leaf mass loss. Moreover, the presence of meiofauna led to strongly increased production of fine particulate organic matter, particularly for treatments with fungi (+637%). The leaf consumption rate by both detritivores tended to decrease in presence of meiofauna. Nonetheless, this effect was only significant at 13 days for the amphipod (-61%), even though a positive effect of meiofauna occurred at 6 days for this detritivore. The lower amphiphod consumption rate likely resulted because of a feeding switch from detritus to meiofauna, evidenced by the strong reduced abundances of cladocerans in this treatment.

5. Our study provides evidence that meiofauna can play a significant role in the detrital food web of streams and rivers. Meiofauna contribute to detrital processing by interacting with microbial communities and macroinvertebrates shredders, which can be positive or negative depending on the trophic relationships. Therefore, meiofauna add complexity to detrital food webs by increasing vertical diversity and modulating biotic interactions.

Key-words: Meiofauna; functional role; leaf breakdown; trophic interactions; aquatic hyphomycete; *Gammarus pulex*; *Sericostoma personatum*.

Introduction

Freshwater meiofauna are typically defined as invertebrates passing through a 0.5 mm sieve (Allan and Castillo 2007), and encompass a diverse group including copepods, nematodes, rotifers, ostracods and cladocerans (Bund & Davids 1993). It is now recognized that the meiofauna compartment contributes significantly to the biodiversity of freshwater ecosystems in terms of species richness and abundance (Robertson, Rundle & Schmid-Araya 2000) and potentially plays a wide range of ecological roles in a variety of ecosystem processes (e.g. Freckman *et al.* 1997; Schmid-Araya & Schmid 2000). Freshwater meiofauna are also known to be abundant and diverse in association with leafy and woody debris (e.g. Golladay & Hax 1995; Casas 1997; Swan & Palmer 2000; Robertson & Milner 2001). Consequently, they may contribute to leaf matter breakdown, although their role is poorly understood.

Aquatic ecosystems receive substantial amounts of allochthonous leaf litter coming from the riparian vegetation along the watershed. These inputs of leaf litter and its breakdown, by providing energy source for biota, play pivotal roles in aquatic food webs (e.g. Cummins *et al.* 1989; Graça 1993; Webster, Wallace & Benfield 1995; Wallace & Webster 1996; Suberkropp 1998). Consequently, leaf breakdown has been recognized as a critical process in the functioning of aquatic environments including streams, littoral zones of lakes, and coastal marine environments (Webster & Benfield 1986; Boulton & Boon 1991) and as such, as a putative indicator of ecosystems integrity (Gessner & Chauvet 2002). Leaf breakdown involves organisms ranging from bacteria and fungi to invertebrate detritivores commonly referred to as shredders (Fig.1A). Bacteria have been rapidly disregarded because the relative importance of bacteria to leaf breakdown may be substantially less than of fungi in terms of standing biomass (Findlay & Arsuffi 1989; Baldy, Gessner & Chauvet 1995). Fungi, mainly aquatic hyphomycetes, contribute to leaf breakdown in two ways: (i) they are responsible for a significant fraction of total leaf breakdown and (ii) fungal colonisation of leaves enhances litter nutritional value and palatability to shredding invertebrates through mycelial biomass and enzymatic metabolization of leaf tissue into more assimilable compounds (e.g. Rounick & Winterbourn 1983; Suberkropp 1998; Bärlocher 2005). Invertebrates have an important effect on the leaf breakdown through direct shredding of leaves and by feeding on fungi, which in turn influences microbial assemblages associated with decaying leaves (Arsuffi & Suberkropp 1989; Suberkropp 1992; Graça 1993).

While numerous studies have documented the role of fungi and shredding invertebrates in leaf breakdown, little information is available regarding the role of meiofauna in the

decomposition process. Due to their abundances, the fauna from this food web compartment may significantly contribute to leaf breakdown. In many ecosystems (e.g. marine, soil and freshwater pelagic systems) meiofauna is already assumed to be a significant component of the heterotrophic assemblage representing a trophic link between detritus, its associated microbial community and larger macroinvertebrates (e.g. Kemp 1990; Golladay & Hax 1995; Robertson, Lancaster & Hildrew 1995; Freckman *et al.* 1997; Hakenkamp & Morin 2000; Schmid-Araya & Schmid 2000; Swan & Palmer 2000). Thus, in their study on the impact of harpacticoid copepod upon detritally associated bacteria, Perlmutter & Meyer (1991) were the first to have designated meiofauna as "microdetritivores" clearly suggesting their role in the detrital dynamics in streams. Previous studies on the role of meiofauna in leaf litter breakdown were focused on temporary meiofauna such as early-instar chironomids and on interactions with microbial communities, notably bacteria associated with decaying leaves. Even if it is not clear if meiofauna feed on detritus itself, such studies reported that lotic meiofauna feed on the associated biofilm (e.g. Fenchel 1970; Hargrave 1972; Meyer-Reil & Faubel 1980). Thus, Palmer et al., (2000) reported an increase in temporary meiofauna abundance (i.e. early instar chironomids, nematodes and oligochaetes) in the leaf packs with highest microbial biomass (i.e. bacteria and fungi). Moreover, it has been shown that meiofauna maydiscriminate between various groups of bacteria and microfungi (e.g. Dash & Cragg, 1972; Carman & Thistle, 1985). Due to their feeding activity, meiofauna may influence microbial assemblages associated with decaying leaves and consequently the quality and availability of organic matter and the microbial-mediated leaf breakdown. Despite this and previous recommendations for more experimental work on this topic (e.g. Palmer *et al.* 1997), no study has so far examined the effects of meiofauna-fungi interactions on the leaf litter breakdown.

Also, there is still a limited knowledge of meiofauna-macrofauna interactions in freshwater ecosystems. Yet, potential trophic relationships between both biological compartments may have important ecological consequences (e.g. influencing macroinvertebrates-mediated leaf breakdown), but the nature of such relationships remains unclear. Lancaster & Robertson (1995) reported the presence of meiofauna in the diet of several predator invertebrates. Only one study reported some meiofauna taxa within the diet of leaf-associated stoneflies (Feminella & Stewart 1986), suggesting trophic relationships between macroinvertebrate shredders and meiofauna associated with leaves. A potential competition between meiofauna and macroinvertebrates shredders for food resources has been proposed in a few studies,

suggesting that meiofauna and macroinvertebrates operate “in parallel” at the primary consumer-level of the food chain (Van de Bund & Davids 1993).

Traditionally, studies on biodiversity effects on leaf litter breakdown have been focused at within-trophic level (i.e. at the litter, microbial or detritivore invertebrates level). However all individuals within an ecosystem are connected via vertical (i.e. complexity across trophic levels) and horizontal (diversity within a trophic level) linkages (Gessner et al., 2010), for example within the food web. Several studies have attempted to integrate the role of diversity variations among trophic levels (i.e. vertical diversity) in ecosystem functioning (e.g. Duffy et al., 2007; Srivastava et al., 2009; Jabiol et al., 2013). These studies, mainly on trophic cascades, have evidenced that changes in vertical diversity could enhance or reduce diversity effects on leaf litter breakdown particularly in the case of predator effects, by altering patterns of competitive dominance among detritivores species. The inclusion of the meiofauna compartment in heterotrophic food web studies (Fig. 1B) may substantially increase the structuration of interactions already known between the microbial, detritivore and predator compartments, and consequently, our understanding of the complex relationships between diversity and ecosystem functioning. In such food web, meiofauna contribution to leaf breakdown may be additive to contribution of others compartments (i.e. fungi and detritivores invertebrates) or non-additive including facilitation (i.e. meiofauna may enhance the contribution of other compartments), resource partitioning (meiofauna may use different food types than detritivores invertebrates) and antagonistic interactions (e.g. competition, predation).

In this study we aimed to evaluate the potential contribution of assemblages of two meiofaunal crustaceans to leaf litter breakdown. We focused on two taxa of permanent meiofauna, one species of cladoceran (i.e. *Chydorus sphaericus*) and one species of cyclopoids *(i.e. Cyclops bohater*), previously observed in leafy detritus collected from headwater forested streams. Similar colonization of leaf packs by microcrustaceans including copepods and cladocerans has been already reported (Gaudes et al. 2009). *C. sphaericus* is the most common of all Cladocera, often attributed to littoral areas near the bottom sediments (e.g.Evans and Stewart, 1997) and macrophyte-dominated habitats (Basinska, 2014). Therefore, *C. sphaericus* is probably better adapted to detrital food sources compared to large-bodied pelagic cladocerans (Vijverberg and Boersma, 1997). *C. sphaericus* has been described as filter feeder for small particles of algae and detritus and scraper feeder (or sweeper) of detritus and diatoms (Fryer, 1968; Lair, 1990; de Eyto, 2000). *C. bohater* is a

cylopoid copepod reported living along the plant-covered banks of stagnant and slow-flowing bodies of water and in the clay-pit (Wierzbicka, 1974). Even if, cyclopoid copepods are abundant in aquatic ecosystems, practically nothing is known of their food and feeding mechanisms. Cyclopoid copepods are able to utilise a much broader food spectrum. Some freshwater cyclopoid copepods are reported predator feeding (e.g. *Macrocyclops albidus*, *Mesocyclops leuckarti*) or herbivorous (e.g. *Eucyclops agilis*, *Acanthocylops bisetosus*) feeding on a variety of algae (e.g. unicellular diatoms, filamentous algae) and protists (Fryer, 1959). They probably switch opportunistically between food sources (crustaceans and rotifers to protists, phytoplankton and bacterial aggregations and detritus) as availability changes within their habitat (Santer et al., 2006).

We designed laboratory experiments on conditioned leaves to test the hypotheses that meiofauna contributes to leaf litter breakdown via interactions with main biological compartments associated with this process i.e. aquatic hyphomycetes and invertebrate detritivores. We predicted that meiofauna would influence fungal assemblages and fungal-mediated leaf breakdown through their feeding activity. Concerning interactions with invertebrate detritivores, we used two shredder species with different feeding strategies i.e. the amphipod *Gammarus pulex* as opportunistic shredder and the trichoptera *Sericostoma personatum* as selective shredder (Colas *et al.* 2013). We tested several hypotheses regarding the role of meiofauna in the breakdown of leaf litter: 1) meiofauna facilitates the decomposition of leaf litter through positive effects on other food web compartments; 2) predation by *G.pulex* dampens the contribution of meiofauna; and 3) competition for resources by meiofauna causes a decrease in the contribution of macroinvertebrates detritivores to leaf breakdown

Materials and methods

Experimental set up

Biological material collection

Senescent alder (*Alnus glutinosa* Gaertn.) leaves were collected from trees just before abscission in autumn 2013 and air-dried in the laboratory. Leaf discs 14 mm in diameter were cut in the alder leaves avoiding the major veins. 76 sets of 25 leaf discs were randomly selected, lyophilised (i.e. “freeze-dried”) and weighted to the nearest 0.01 mg. Each set of leaf discs was enclosed into a mesh bag (10 x 10 cm, 500 µm mesh size). Leaf bags were then incubated in a reference forested headwater stream located in the Pyrenees Mountains (south-west France) to allow fungal colonisation. Bags were retrieved after 15 days, returned to the

laboratory and rinsed with stream water to remove fine particulate matter. The remaining leaf material after conditioning stage of 4 sets of 25 leaf discs was preserved at -20°C and later lyophilised and weighed to the nearest 0.01 mg to estimate the initial dried mass introduced in the experimental units. 30 L of water was collected for the laboratory experiments, filtered (Whatman®, 0.45 µm pore size) and kept at 4°C in the dark until use.

The selection of meiofauna taxa has been performed based on previous observations on meiofauna assemblages identified from leaves samples collected from three streams located in the Pyreneees Mountains. Two taxa were chosen according their abundance within leaves samples and their resistance to laboratory conditions: *Chydorus sphaericus* (O.F. Müller, 1976; cladocerans) and *Cyclops bohater* (Kozminski, 1933; copepods) named hereafter *Chydorus* and *Cyclops*, respectively. Organisms of both taxa were sampled using a plankton net (50 µm mesh size) in two ponds near the laboratory. The ponds are situated in a forest, and hence contain high amounts of leaf litter as well as high abundance of meiofauna, which allows collecting more individuals than in streams. Organisms were individually sorted and counted in the laboratory. Ovigerous females were systematically removed to avoid potential nesting. Then, 36 sets of 20 (± 3) individuals *Cyclops* and 40 (± 4) *Chydorus* were randomly selected and starved prior to the experiment by putting them in plastic container with 500 ml of filtered stream water at 10°C without food during 24h.

Two species of shredder invertebrates with different feeding habits were used: the amphipod *Gammarus pulex* (L.) and the trichoptera *Sericostoma personatum* (Spence), named hereafter *Gammarus* and *Sericostoma*, respectively. Individuals were sampled in a reference headwater stream located in the Pyreneees Mountains, sorted and counted at the laboratory. Particular attention was taken to select individuals of a same size class (8-9 mm for *Gammarus*; 10-11mm for *Sericostoma*). For *Gammarus*, females were systematically removed. Individuals were then starved prior to the experiment by putting them in plastic container with 500 ml of filtered stream water at 10°C without food for 24 h.

Experimental design

Contribution of meiofauna to leaf breakdown were evaluated by using feeding assays (Elger and Lemoine, 2005; Colas et al., 2016). Six treatments were used (Table 1) and two incubation times (i.e. 6 and 13 days). For each treatment and incubation time, six replicates were realized using a permuted block randomisation (detailed on block randomisation is available in the Supporting information, Appendix A). For each replicate, one set of 25 leaf

discs was introduced into a plastic container with 300 ml of filtered water. For treatments with shredder invertebrates, one individual of *Gammarus* (i.e. FG and FGM) or *Sericostoma* (i.e. FS and FSM) was randomly assigned to experimental unit. Similarly, for treatments with meiofauna (i.e. FM, FGM and FSM), one set of organisms was randomly assigned to treatments with meiofauna and experimental unit. The 72 experimental units were placed for 6 and 13 days in a thermostatic chamber (10 ± 1°C) under a daily photoperiod of 10 h light and 14 h dark. At the end of each experiment, for each replicate, ten leaf discs were randomly selected. Five discs were immediately introduced into Erlenmeyer flasks containing 20 mL of filtered water for the sporulation experiment (see section 2.2.4). Five discs were preserved at -80°C until ergosterol analysis. The remaining leaf discs were preserved at -20°C and later lyophilised prior to weighing. Then, meiofauna organisms were removed individually from leaves and counted in order to determine survival and preserved in 4% formalin. Invertebrates were frozen at -20°C and later lyophilised.

Variables measured

Leaf litter decomposition

For each replicate, the remaining exposed discs were lyophilised and weighed to the nearest 0.01 mg. The weights of leaf discs used for ergosterol analysis and sporulation experiment were added to the remaining mass of the corresponding set of leaf discs. Leaf litter decomposition was expressed as the percentage of mass remaining after subtracting fungal biomass (as estimated by ergosterol contents, see Section 2.2.5). Additivity between meiofauna and macrofauna was tested for each shredder specie by extracting the contribution of each compartment coming from treatment FG and FS (i.e. the loss of dry mass, expressed in percentage) and by comparing the sum of each contribution to the joint contribution coming from treatments FGM and FSM. To formulate this hypothesis, if there is additivity between compartments it means that:

(1)

$$LDM_{FGM\ or\ FSM} = IC_{Fungi} + IC_{meiofauna} + IC_{macrofauna}$$

with:

$$IC_{Fungi} = LDM_F$$

$$IC_{mesofauna} = LDM_{FM} - LDM_F$$

$$IC_{macrofauna} = LDM_{FG\ or\ FS} - LDM_F$$

Where LDM is the loss of dry mass corresponding to each treatment.

Consumption rates

At the end of the experiments, invertebrates were weighed to the nearest 0.01 mg and relative consumption rates (RCR), expressed in mg leaf mg invertebrate^{-1} day^{-1}, were calculating following Maltby (1992) using Eq.(2):

(2)

$$\text{RCR} = \frac{[(DM_1 \times C) - DM_2]}{W \times T}$$

where DM_1 is the initial mass of the leaf discs (in mg) exposed to invertebrates, DM_2 the remaining weight of the leaf discs (in mg) at the end of the experiments, W the dry weight of invertebrates (in mg) and T the exposure time (in days). For each treatment, the initial weight of leaves was corrected for non-consumptive weight loss by a correction factor (C) according Eq. (3):

(3)

$$\text{C} = \frac{\sum(DM_b / DM_a)}{6}$$

where DM_a and DM_b are the initial and final dry weights (mg) of control leaf discs used to estimate the mass loss not caused by invertebrate consumption. For treatment FG and FS (i.e. fungi x invertebrate), leaf discs of treatment F (i.e. fungi) were used as control. For treatment FGM and FSM (i.e. fungi x meiofauna x invertebrate), leaf discs of treatment FM (i.e. fungi x meiofauna) were used as control.

Fine particulate organic matter (FPOM) production

At the end of experiments, water from the experimental units (i.e. 300 ml) was filtered on a 0.45 µm pore size, 25 mm diameter, nitrate cellulose membrane (Whatman®). The membrane was first washed with pure water, dried at 80°C and weighed to the nearest 0.001 mg. After filtration, the membrane was dried at 80°C for 12 h and weighed to determine the mass of FPOM produced.

Fungal assemblages

Erlenmeyer flasks containing five discs and 20 mL of filtered water were placed on an orbital shaker (100 rpm) for 48h at 10°C to induce fungal sporulation. After incubation, the leaf discs were removed and the conidial suspension was poured into 50-ml centrifuge tube, which was rinsed in the flask with distilled water (3 x 2 ml) to dislodge remaining attached conidia. All rinse water was collected in the tube, and the volume was adjusted to 30 ml with distilled water and 2 ml of 37% formalin. Conidial suspensions were stored in the dark until analysis. The five leaf discs were frozen at -20°C, lyophilised and then weighed to the nearest 0.01 mg. Before conidial identification, Triton X-100 solution (5%) was added to the suspensions, which were then shaken on a magnetic stirrer for 10 min to ensure a uniform distribution of conidia. An aliquot of the suspensions was filtered (membrane filter, 5 µm porosity, 25 mm diameter, Millipore, Bedford, MA, USA), stained with Trypan blue (0.1% in 60% lactic acid), counted and identified under microscope at 200-400x (Bärlocher, 2005 ; Gulis et al. 2005). For each species, the sporulation rate (conidia mg^{-1} leaf day^{-1}) was determined.

Fungal biomass

Ergosterol was extracted from leaf discs and quantified as previously described (Gessner and Schmitt, 1996). Briefly, the leaf discs were lyophilised, weighed to the nearest 0.1 mg and heated in 5 ml of alkaline methanol (KOH, 8 g L^{-1}) for 30 min at 80°C. The extract was purified by solid-phase extraction on cartridges (Waters Oasis HLB, 60 mg, cm^3). Ergosterol was separated by reversed phase high performance liquid chromatography on C18 and quantified by measuring absorbance at 282 nm. Ergosterol was converted to fungal biomass using a conversion factor of 5.5 mg ergosterol g^{-1} mycelial dry mass (Gessner & Chauvet 1993).

Data analyses

Multiple comparisons were performed to test the hypotheses stated in the introduction concerning interactions between meiofauna and fungi or invertebrates compartments. Leaf litter breakdown and FPOM production have been compared between treatments F and FM (fungi-meiofauna hypothesis), treatments FG and FGM or treatments FS and FSM (invertebrates-meiofauna hypotheses) using analyses of covariance (ANCOVA). Two-way analyses of variance (ANOVA) have been used to compare fungal biomass, conidia production and relative consumption rates between treatments with and without meiofauna. At the end of experiments, meiofauna abundance has been compared between experimental

units with and without macrofauna using Student's t-test in order to test the hypothesis of invertebrate predation on meiofauna. Additivity between meiofauna and invertebrates has been tested by comparing leaf litter breakdown of treatments (FGM and FSM) and the sum of independent contribution of each compartment (IC) calculated for each replicate coming from treatments F, FM, FG and FS using Student's t-test. For all parametric analyses, normal distribution and homoscedasticity were respected. R software (R Development Core Team 2008) was used for all statistical analyses.

Results

Leaf mass loss

The dry mass remaining of alder leaves was significantly lower (Fig. 2; $F_{3,20} = 16.7$, $p < 0.01$) in the without-detritivore treatment with meiofauna (FM) than in the treatment without meiofauna (F), with percentage remaining at 13 d being 66% and 79%, respectively. The remaining dry mass was not different between treatments with (FGM) and without (FG) meiofauna in presence of *Gammarus* (Fig. 2; 76 and 75 %, respectively). Conversely, when *Sericostoma* was present, the dry mass remaining was significantly lower (Fig. 2; $F_{3,20} = 23.6$, $p < 0.001$) in treatment with meiofauna (FSM; 56%) than without (FS; 64%).

FPOM production

The amount of FPOM released was significantly higher (Fig. 2; $F_{3,20} = 29.7$, $P < 0.001$) in treatments with meiofauna (FM: 0.017 and 0.028 mg at 6 d and 13 d respectively) compared to the treatment with fungi alone (F: 0.0036 and 0.0038 at 6 d and 13 d, respectively). Similarly, when *Gammarus* was present, the amount of FPOM released was significantly higher (Fig. 2; $F_{3,20} = 3.3$, $P < 0.05$) in treatment with meiofauna (FGM; 0.028 mg) than without (FG; 0.017), particularly at 6 d. In contrast, the FPOM production was not different between treatments without (FS: 0.032 and 0.045 mg at 6 d and 13 d, respectively) and with meiofauna (FSM: 0.043 and 0.047 mg at 6 d and 13 d, respectively) for *Sericostoma* (Fig. 2).

Leaf consumption by detritivores

The relative consumption rates of *Gammarus* showed a non-significant increase in presence of meiofauna at 6 d, but was significantly depressed by 61% under the effect of meiofauna at 13 d (Fig. 3A; $F_{3,21} = 17.8$, $p < 0.01$). The relative consumption rates of *Sericostoma* were not significantly different between treatments even though a slight decrease in presence of meiofauna was observed at both 6 and 13 d (Fig. 3B).

Fungal community structure, activity and biomass

The fungal richness based on sporulating species varied between 3 and 4. The dominant species was *Alatospora acuminata* Ingold accounting for a total of 92.2 (±3) % on average, with *Tetraladium marchalanium* De Wild, *Heliscus ludunensis* Saccardo & Therry and *Tetracladium setigerum* (Grove) Ingold being less abundant. The total sporulation rates were not different between treatments (Fig. 4). Except for *T. marchalianum* (TEMA) for which sporulation rates were significantly lower in treatment with meiofauna than with fungi only ($F_{3,21}$ =4.3, $p < 0.01$), sporulation rates of species present did not differ between treatments. In absence of detritivores, mycelial biomass was significant higher in treatments with meiofauna compared to treatment without at 13 d (Fig. 4; $F_{3,21}$ =11.8, $p < 0.01$). The presence of both *Gammarus* and meiofauna induced a significantly lower mycelial biomass (Fig. 4; $F_{3,21}$ =18.6, $p < 0.01$) while no difference were found in presence of both *Seriscostoma* and meiofauna (Fig. 4).

Meiofauna survival

At both 6 (t (9.84 df) = 7.20, $p < 0.001$) and 13 days (t (9.81 df) = 17.3, $p < 0.001$), the abundance of *Chydorus* was significantly lowered by the presence of *Gammarus* (Fig. 5A). The abundance of *Chydorus* was not significantly affected by the presence of *Sericostoma* at 6 (Fig. 5A; t (7.45 df) = 1.88, $p > 0.05$) and 13 days (Fig. 5A; t (9.9 df) = -0.26, $p > 0.05$). The abundance of *Cyclops* did not significantly differ between treatments with and without *Gammarus* at 6 days (Fig.5B; t (9.90 df) = 2.21, $p = 0.05$) but was significantly different between these two treatments at 13 days (Fig. 5B; t (9.9 df) = 2.7, $p = 0.02$). Like for *Chydorus*, the abundance of *Cyclops* was not significantly affected by the presence of *Sericostoma* at 6 (Fig.5B; t (5.45 df) = 0.67, $p > 0.05$) and 13 days (Fig.5B; t (6.34 df) = 1.50, $p > 0.05$). Table 2 summarizes the main results about comparisons between treatments without or with meiofauna.

Functional additivity

The sum of individual contributions (IC) of *Gammarus* and meiofauna to litter breakdown did not significantly differ from the breakdown when all decomposers occurred together (JC) at 6 d (Fig. 5A; t (7.33 df) = 1.15, $p > 0.05$). This also held for *Sericostoma* at 6 days (Fig. 5B; t (9.5 df) = 0.12, $p > 0.05$) and at 13 days (Fig. 5B; t (7.6 df) = 0.5, $p > 0.05$), suggesting additivity between meiofauna and *Sericostoma*. Conversely, IC and JC were significantly different for treatments with *Gammarus* at 13 days (Fig. 4A; t(5.8 df) = 3.15, $p < 0.05$).

Indeed, the sum of individual contributions of *Gammarus* and the other decomposers to litter breakdown (IC) was significantly higher than breakdown when all decomposers occurred together at 13 d suggesting no additivity between meiofauna and *Gammarus*.

Discussion

Our study is the first to provide experimental evidence of direct and indirect interactions between meiofauna and the two main decomposer compartments, i.e. macroinvertebrates and fungi, and the consequences of such interactions on the leaf litter breakdown. Indeed, the presence of meiofauna enhanced leaf breakdown mediated by leaf-shredding invertebrates and fungi by 22% and 62% respectively, and consequently led to increased food availability (via FPOM) to other organisms (e.g. collector invertebrates). While meiofauna contribute to leaf breakdown, their role varied with the presence of two leaf-shredding invertebrates species due to complex trophic interactions such as resource switching and complementarity.

The reduced leaf mass in treatment with meiofauna compared to treatment with fungi alone can be related to direct consumption of leaves by meiofauna or alternatively meiofauna may facilitate greater fungal biomass by bioturbating the leaf surface or by preferentially consuming bacteria, thereby reducing competition for resources. While there is no clear evidence than meiofauna feed directly on leaf material, some studies reported their feeding preference for small particles of detritus and FPOM-associated microflora (Perlmutter and Meyer, 1991; Sherr & Sherr 1994; Snyder & Hoch 1996; Mikola & Setälä 1998; Ribblett *et al.* 2005; Basinska et al. 2014). For instance, in a laboratory experiment on the response of three chydorid species to food, de Eyto and Irvine (2001) evidenced that *C.sphaericus* populations are able to utilise detritus (i.e. FPOM) for growth and reproduction. Some studies emphasized that meiofauna may enhance detritus mineralization presumably due to high rates of bacterial turnover in response to grazing pressure by consumers (e.g. Perlmutter & Meyer, 1991; Sherr & Sherr, 1994; Snyder & Hoch, 1996; Mikola & Setälä, 1998). However, nothing is known about the feeding pressure of meiofauna on leaf-associated fungi while fungi are clearly the predominant microbes on coarse particulate organic material (i.e. leaf litter and woody debris) in streams (Findlay et al. 2002). In our study, the feeding effect of meiofauna led to an increased fungal biomass despite no significant change in fungal richness. In addition, sporulation rates of *Tetracladium marchalianum* were significantly depressed by 35% on average in presence of meiofauna, suggesting that meiofauna specifically affected some fungal species, either directly or indirectly. This result raises questions about feeding preferences of meiofauna, while more information on the diet and feeding behaviour of

meiofauna is still needed. Nonetheless, few studies have already reported some species of meiofauna to be able to discriminate among various groups of microfungi (e.g. Dash & Cragg 1972; Carman & Thistle 1985). The selective feeding of leaf-shredding invertebrates on leaf-colonizing fungi has been abundantly documented (e.g. Arsuffi & Suberkropp 1989; Graça, Maltby & Calow 1994; Rong, Sridhar & Bärlocher 1995). That such feeding preferences on fungi also occur in meiofauna species is expectable. This nevertheless deserves to be confirmed by additional laboratory studies as *Tetracladium marchalianum* was overall marginally present on the leaf discs in contrast to *Alatospora acuminata* but whose sporulation rate did not differ in presence of meiofauna. Another important question, not dealt with here, is whether meiofauna choose food items to fulfil specific nutrient requirements (Hakenkamp & Morin 2000). Fungal conditioning and nutrient content enrichment (i.e. P content) of decaying leaves are known to positively influence macroinvertebrate survival and/or growth rates (e.g. Maltby 1999; Graça et al. 2001; Danger et al. 2013). Whilst studies on this topic are still rare, some research has suggested similar mechanisms such as significant effects of biofilm composition and quality of organic matter on copepod reproduction (Brown et al. 2003) and on leaf-associated meiofauna assemblages (e.g. Lenting, Williams & Fraser 1997; Palmer et al. 2000).

The co-occurrence of meiofauna and the opportunistic shredder *Gammarus* (Usseglio-Polatera *et al.* 2000; Colas *et al.* 2013) did not induce any significant change in leaf breakdown. Nonetheless, the omnivorous Gammarus unsurprisingly switched food resources in the presence of meiofauna, with the resulting predation leading to drastically lower densities of the cladoceran *C. sphaericus*. Several studies already reported the herbivore/predator plasticity of *Gammarus* spp. (e.g. MacNeil, Dick & Elwood 1997; Felten *et al.* 2008; Colas *et al.* 2014), probably linked to its feeding apparatus able of coping with a wide variety of foodstuffs (MacNeil *et al.* 1997). Previous studies have shown predation of *Gammarus* spp on meiofauna, particularly cladoceran species (Hutchinson 1937; Kortelainen 1991). Surprisingly, the reduced leaf consumption by *Gammarus* did not lead to slower leaf breakdown, likely due to compensatory mechanisms that can dampen or even reverse the top-down predator effects predicted by the trophic cascade concept (Gessner *et al.* 2010; Majdi *et al.* 2014). For instance, microbial decomposers might process litter more efficiently once released from grazing pressure by leaf-shredding macroinvertebrates (e.g. Mancinelli, Costantini & Rossi 2002). Conversely, in the presence of the caddisfly Sericostoma, the meiofauna were able to enhance breakdown rates. Despite increased leaf mass loss, FPOM

production did not change suggesting that the meiofauna may use this resource when a non-predatory shredder is also consuming the same source of detritus (resource partitioning). The complex trophic interactions between meio- and macrofauna as described above may thus have important consequences for the way organic matter is transferred through the food webs. While some authors have proposed that meio- and macrofauna may operate "in series" in a linear food chain (Strayer 1991) or "in parallel" at the primary consumer level of the food chain (Van de Bund & Davids 1993), our study shows that the nature of interactions between meio- and macrofauna and their impact on ecosystem processes are species-dependent. In in-stream conditions, where the species mixture and their interactions are more important than in our experiments, this species dependency probably leads to even more complex trophic webs.

This paper has focused on the role of meiofauna in leaf breakdown. This process plays a pivotal role in food webs of a wide variety of aquatic ecosystems and, as such, has been identified as a putative indicator of ecosystem integrity (e.g. Wallace & Webster 1996; Gessner & Chauvet 2002; Hieber & Gessner 2002). Although involved mechanisms need to be further explored by additional experiments, results of our study provides evidence that meiofauna may contribute to leaf breakdown probably by trophic interactions with leaf-decaying microbial and macroinvertebrates biota. In this present paper, we have chosen to use a controlled laboratory setting because this study was designed to test the hypothesis that meiofauna could alter leaf breakdown mediated by fungi and leaf-shredding invertebrates. Nonetheless, trophic interactions identified in this study may not occur or to less extent in-field conditions. For instance, top-down effects of gammarids on meiofauna may be dampen in field conditions by the presence of refugia habitats for meiofauna and the availability of prey alternatives for gammarids. Thus, further experiments are need under laboratory and in-stream conditions to increase understanding of organic matter processing and trophic interactions between all compartments involved in this process. More specifically, a greater appreciation for the contribution of meiofauna may help to improve methods assessing detrital processing in the field and experimental mesocosms. Currently, the arbitrary mesh sizes typically used in the assessment of leaf breakdown rates based on leaf bags (e.g. Graça et al., 2005) may obfuscate the relative importance of microbes and meiofauna detritivores to rates of leaf breakdown. To cover this gap, a third type of leaf bags with an intermediate mesh size (e.g. from 500 to 1000 µm) could be proposed. Such an approach should aim to identify meiofauna taxa assemblages and determine the extent of their colonization of leaf surfaces together with their contribution to leaf breakdown rate as already suggested by Gaudes et al.

(2009). In addition, a comparison of the role of meiofauna between various aquatic ecosystems should provide interesting insights with, for instance, an increased contribution of meiofauna being expected in lentic ecosystems such as lakes, pools and downstream reaches of streams and rivers.

Conclusion

Results of this study provide evidence that meiofauna contribute to the detrital process in aquatic ecosystems. Although the mechanisms involved need to be elucidate by further experiments, meiofauna may facilitate fungal-mediated breakdown in addition to their own detrital consumption. This study evidences for complex trophic interactions between meiofauna and leaf-shredding invertebrates (i.e., facilitation, resource partitioning and /or predation) suggesting that the role of meiofauna to leaf breakdown varied according the presence of different shredder species. Therefore, meiofauna can change the way energy from organic matter moved through the food web depending on trophic relationships with the microbial and macrofaunal assemblages associated with decaying leaves. Because the meiofauna compartment increases the food web complexity of heterotrophic assemblages, their consideration is crucial for a comprehensive understanding of organic matter and nutrients dynamics in aquatic ecosystems.

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References

Arsuffi, T.L. & Suberkropp, K. (1989) Selective feeding by shredders on leaf-colonizing stream fungi: comparison of macroinvertebrate taxa. *Oecologia*, **79**, 30–37.

Baldy, V., Gessner, M.O. & Chauvet, E. (1995) Bacteria, fungi and the breakdown of leaf litter in a large river. *Oikos*, 93–102.

Bärlocher, F. (2005) Freshwater fungal communities. *The fungal community. Its organization and role in the ecosystem*, CRC pp. 39–59. Dighton J, White JF, Oudemans P, Boca Raton.

Boulton, A.J. & Boon, P.I. (1991) A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf? *Marine and Freshwater Research*, **42**, 1–43.

Brown, R.J., Rundle, S.D., Hutchinson, T.H., Williams, T.D. & Jones, M.B. (2003) Small-scale detritus-invertebrate interactions: influence of detrital biofilm composition on development and reproduction in a meiofaunal copepod. *Archiv für Hydrobiologie*, **157**, 117–129.

Bund, W.J. & Davids, C. (1993) Complex relations between freshwater macro-and meiobenthos: interactions between Chironomus riparius and Chydorus piger. *Freshwater Biology*, **29**, 1–6.

Van de Bund, W.J. & Davids, C. (1993) Complex relations between freshwater macro- and meiobenthos: interactions between Chironomus riparius and Chydorus piger. *Freshwater Biology*, **29**, 1–6.

Carman, K.R. & Thistle, D. (1985) Microbial loop partitioning by three species of betnhic copepods. *Marine Biology*, 230–240.

Casas, J.J. (1997) Invertebrate assemblages associated with plant debris in a backwater of a mountain stream: natural leaf packs vs. debris dam. *Journal of Freshwater Ecology*, **12**, 39–49.

Chauvet, E. (1987) Changes in the chemical composition of alder, poplar and willow leaves during decomposition in a river. *Hydrobiologia*, **148**, 35–44.

Colas, F., Baudoin, J.-M., Danger, M., Usseglio-Polatera, P., Wagner, P. & Devin, S. (2013) Synergistic impacts of sediment contamination and dam presence on river functioning. *Freshwater Biology*, **58**, 320–336.

Colas, F., Vigneron, A., Felten, V. & Devin, S. (2014) The contribution of a niche-based approach to ecological risk assessment: Using macroinvertebrate species under multiple stressors. *Environmental Pollution*, **185**, 24–34.

Cummins, K.W., Wilzbach, M.A., Gates, D.M., Perry, J.B. & Taliaferro, W.B. (1989) Shredders and riparian vegetation. *BioScience*, 24–30.

Danger, M., Arce Funck, J., Devin, S., Heberle, J. & Felten, V. (2013) Phosphorus content in detritus controls life-history traits of a detritivore. *Functional Ecology*, **27**, 807–815.

Dash, M.C. & Cragg, J.B. (1972) Selection of microfungi by Enchytraeidae (Oligochaeta) and other members of the soil fauna. *Pedologia*, **12**, 282–286.

Duffy, J.E., Cardinale, B.J., France, K.E., McIntyre, P.B., Thébault, E. & Loreau, M. (2007) The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology Letters*, **10**, 522–538.

Evelyn Hutchinson, G. (1937) Limnological studies in Indian Tibet. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, **35**, 134–177.

Felten, V., Tixier, G., Guérold, F., De Crespin, D.B. & Dangles, O. (2008) Quantification of diet variability in a stream amphipod: implications for ecosystem functioning. *Fundamental and Applied Limnology/Archiv für Hydrobiologie*, **170**, 303–313.

Feminella, J.M. & Stewart, K.W. (1986) Diet and predation by three leaf-associated stoneflies (Plecoptera) in an Arkansas mountain stream. *Freshwater Biology*, **16**, 521–538.

Fenchel, T. (1970) Studies on the decomposition of organic detritus derived from the turtlegrass Thalassia testudinum. *Limnology and Oceanography*, **15**, 14–20.

Findlay, S.E.G. & Arsuffi, T.L. (1989) Microbial growth and detritus transformations during decomposition of leaf litter in a stream. *Freshwater Biology*, **21**, 261–269.

Findlay, S., Tank, J., Dye, S., Valett, H.M., Mulholland, P.J., McDowell, W.H., Johnson, S.L., Hamilton, S.K., Edmonds, J., Dodds, W.K. & others. (2002) A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. *Microbial Ecology*, **43**, 55–66.

Freckman, D.W., Blackburn, T.H., Brussaard, L., Hutchings, P., Palmer, M.A. & Snelgrove, P.V. (1997) Linking biodiversity and ecosystem functioning of soils and sediments. *Ambio*, 556–562.

Gaudes, A., Artigas, J., Romani, A.M., Sabater, S. & Munoz, I. (2009) Contribution of microbial and invertebrate communities to leaf litter colonization in a Mediterranean stream. *Journal of the North American Benthological Society*, **28**, 34–43.

Gessner, M.O. & Chauvet, E. (1993) Ergosterol-to-Biomass Conversion Factors for Aquatic Hyphomycetes. *Applied and Environmental Microbiology*, 502–507.

Gessner, M.O. & Chauvet, E. (2002) A case for using litter breakdown to assess functional stream integrity. *Ecological applications*, **12**, 498–510.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H. & Hättenschwiler, S. (2010) Diversity meets decomposition. *Trends in Ecology & Evolution*, **25**, 372–380.

Golladay, S.W. & Hax, C.L. (1995) Effects of an engineered flow disturbance on meiofauna in a north Texas prairie stream. *Journal of the North American Benthological Society*, 404–413.

Graça, M.A.S. (1993) Patterns and processes in detritus-based stream systems. *Limnologica*, 107–114.

Graça, M.A.S., Cressa, C., GESSNER, T., Feio, M.J., Callies, K.A. & Barrios, C. (2001) Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshwater Biology*, **46**, 947–957.

Graça, M. a. S., Maltby, L. & Calow, P. (1994) Comparative ecology of Gammarus pulex (L.) and Asellus aquaticus (L.) II: fungal preferences. *Hydrobiologia*, **281**, 163–170.

Hakenkamp, C.C. & Morin, A. (2000) The importance of meiofauna to lotic ecosystem functioning. *Freshwater Biology*, **44**, 165–175.

Hargrave, B.T. (1972) Aerobic decomposition of sediment and detritus as a function of particle surface area and organic content. *Limnology and Oceanography*, **17**, 583–586.

Hieber, M. & Gessner, M.O. (2002) Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, **83**, 1026–1038.

Jabiol, J., McKie, B.G., Bruder, A., Bernadet, C., Gessner, M.O. & Chauvet, E. (2013) Trophic complexity enhances ecosystem functioning in an aquatic detritus-based model system. *Journal of Animal Ecology*, n/a–n/a.

Kemp, P.F. (1990) The fate of benthic bacterial production. *Reviews in Aquatic Sciences*, **2**, 109–124.

Kortelainen, I. (1991) Gammarus lacustris: herbivore or predator? *Annales Universitatis Turkuensis Series A II Biologica-Geographica-Geologica*, **0**, 31–34.

Lancaster, J. & Robertson, A.L. (1995) Microcrustacean prey and macroinvertebrate predators in a stream food web. *Freshwater Biology*, **34**, 123–134.

Lecerf, A., Hampel, H., Yu, H., Nijs, J., Hellings, L. & Tackx, M. (2008) Decomposition of willow (Salix triandra L.) leaves in the Schelde estuary (Belgium).

Lenting, N., Williams, D.D. & Fraser, B.G. (1997) Qualitative differences in interstitial organic matter and their effect on hyporheic colonisation. *Hydrobiologia*, **344**, 19–26.

Lillebø, A.I., Flindt, M.R., Pardal, M.Â. & Marques, J.C. (1999) The effect of macrofauna, meiofauna and microfauna on the degradation of Spartina maritima detritus from a salt marsh area. *Acta Oecologica*, **20**, 249–258.

MacNeil, C., Dick, J.T. & Elwood, R.W. (1997) The trophic ecology of freshwater Gammarus spp.(Crustacea: Amphipoda): problems and perspectives concerning the functional feeding group concept. *Biological Reviews*, **72**, 349–364.

Majdi, N., Boiché, A., Traunspurger, W. & Lecerf, A. (2014) Predator effects on a detritus-based food web are primarily mediated by non-trophic interactions. *Journal of Animal Ecology*, **83**, 953–962.

Maltby, L. (1999) Studying Stress: The Importance of Organism-Level Responses. *Ecological Applications*, **9**, 431–440.

Mancinelli, G., Costantini, M. & Rossi, L. (2002) Cascading effects of predatory fish exclusion on the detritus-based food web of a lake littoral zone (Lake Vico, central Italy). *Oecologia*, **133**, 402–411.

McIntyre, A.D. (1969) Ecology of marine meiobenthos. *Biological Reviews*, **44**, 245–290.

Meyer-Reil, L.A. & Faubel, A. (1980) Uptake of organic matter by meiofauna organisms and interrelationships with bacteria. *Marine Ecology Progress in Series*, **3**, 251–256.

Michael Gee, J. & Somerfield, P.J. (1997) Do mangrove diversity and leaf litter decay promote meiofaunal diversity? *Journal of Experimental Marine Biology and Ecology*, **218**, 13–33.

Mikola, J. & Setälä, H. (1998) No evidence of trophic cascades in an experimental microbial-based soil food web. *Ecology*, **79**, 153–164.

Palmer, M., Covich, A., Finlay, B., Gilbert, J., Hyde, K., Johnson, R., Kairesala, T., Lake, P., Lovell, C., Naiman, R. & others. (1997) Biodiversity and ecosystem processes in freshwater sediments. *Ambio*, 571–577.

Palmer, M.A., Swan, C.M., Nelson, K., Silver, P. & Alvestad, R. (2000) Streambed landscapes: evidence that stream invertebrates respond to the type and spatial arrangement of patches. *Landscape Ecology*, **15**, 563–576.

Perlmutter, D.G. & Meyer, J.L. (1991) The impact of a stream-dwelling harpacticoid copepod upon detritally associated bacteria. *Ecology*, 2170–2180.

Petersen, R.C. & Cummins, K.W. (1974) Leaf processing in a woodland stream*. *Freshwater biology*, **4**, 343–368.

Poulson-Ellestad, K., Mcmillan, E., Montoya, J. P., & Kubanek, J. (2014). Are offshore phytoplankton susceptible to Karenia brevis allelopathy?. Journal of Plankton Research, 36(5), 1344-1356.

R Development Core Team. (2008) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ribblett, S.G., Palmer, M.A. & Wayne Coats, D. (2005) The importance of bacterivorous protists in the decomposition of stream leaf litter. *Freshwater Biology*, **50**, 516–526.

Robertson, A.L., Lancaster, J. & Hildrew, A.G. (1995) Stream hydraulics and the distribution of microcrustacea: a role for refugia? *Freshwater Biology*, **33**, 469–484.

Robertson, A.L. & Milner, A.M. (2001) Coarse Particulate Organic Matter: A habitat or food resource for the meiofaunal community of a recently formed stream? *Archiv Fur Hydrobiologie*, **152**, 529–541.

Robertson, A.L., Rundle, S.D. & Schmid-Araya, J.M. (2000) Putting the meio-into stream ecology: current findings and future directions for lotic meiofaunal research. *Freshwater Biology*, **44**, 177–183.

Rong, Q., Sridhar, K.R. & Bärlocher, F. (1995) Food selection in three leaf-shredding stream invertebrates. *Hydrobiologia*, **316**, 173–181.

Rounick, J.S. & Winterbourn, M.J. (1983) Leaf processing in two contrasting beech forest streams: effects of physical and biotic factors on litter breakdown. *Archiv fur hydrobiology*.

Schmid-Araya, J.M. & Schmid, P.E. (2000) Trophic relationships: integrating meiofauna into a realistic benthic food web. *Freshwater Biology*, **44**, 149–163.

Sherr, E.B. & Sherr, B.F. (1994) Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. *Microbial Ecology*, **28**, 223–235.

Snyder, R.A. & Hoch, M.P. (1996) Consequences of protist-stimulated bacterial production for estimating protist growth efficiencies. *Hydrobiologia*, **341**, 113–123.

Srivastava, D.S., Cardinale, B.J., Downing, A.L., Duffy, J.E., Jouseau, C., Sankaran, M. & Wright, J.P. (2009) Diversity has stronger top-down than bottom-up effects on decomposition. *Ecology*, **90**, 1073–1083.

Strayer, D.L. (1991) Perspectives on the size structure of lacustrine zoobenthos, its causes, and its consequences. *Journal of the North American Benthological Society*, 210–221.

Suberkropp, K. (1992) Interactions with invertebrates. *The ecology of aquatic hyphomycetes*, Springer-Verlag pp. 118–134. F. Bärlocher, Berlin.

Suberkropp, K. (1998) Microorganisms and organic matter processing. *River ecology and management: lessons from the Pacific Coastal Ecoregion.*, Springer pp. 120–143. Naiman RJ, Bilby RE, Berlin.

Swan, C.M. & Palmer, M.A. (2000) What drives small-scale spatial patterns in lotic meiofauna communities? *Freshwater Biology*, **44**, 109–121.

Usseglio-Polatera, P., Bournaud, M., Richoux, P. & Tachet, H. (2000) Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. *Freshwater Biology*, **43**, 175–205.

Wallace, J.B. & Webster, J.R. (1996) The role of macroinvertebrates in stream ecosystem function. *Annual review of entomology*, **41**, 115–139.

Webster, J.R. & Benfield, E.F. (1986) Vascular plant breakdown in freshwater ecosystems. *Annual review of ecology and systematics*, 567–594.

Webster, J.R., Wallace, J.B. & Benfield, E.F. (1995) Organic processes in streams of the eastern United States. *Ecosystems of the world 22, River and stream ecosystems*, Elsevier pp. 117–117. C.E. Cushing, K.W. Cummins, and G.W. Minshall, New York, USA.

Rôle du méso-zooplancton dans un estuaire en voie de restauration : l'Escaut.

Résumé : L'estuaire de l'Escaut est un estuaire en voie de restauration. L'étude s'intéresse à l'écologie de la communauté zooplanctonique dans le tronçon d'eau douce de l'Escaut, ou, suite à l'amélioration de la qualité de l'eau le copépode calanoide *Eurytemora affinis* est devenu dominant depuis 2007 et les copépodes cyclopoïdes ont diminués en abondance.

Nous avons cherché à trouver quels facteurs environnementaux expliquent ce changement de la composition de la communauté zooplanctonique. Les résultats d'analyses RDA et GLM entre les abondances des taxons zooplanctoniques et les facteurs environnementaux montrent un lien étroit entre l'augmentation de l'abondance d'*E. affinis* et l'augmentation des concentrations en oxygène ainsi que la diminution des concentrations en $N\text{-}NH_4$. En fait, le tronçon amont de l'estuaire est devenu 'permissive' pour le développement d'*E. affinis* à partir ou la concentration en O_2 a dépassé le seuil de 4 mg L^{-1} et la concentration en $N\text{-}NH_4$ est resté en dessous de 2 mg L^{-1}. La cause du déclin en abondance des cyclopoids reste à trouver.

Dans l'Escaut, le phytoplancton est fortement dominé par les diatomées, mais la concentration en Si dissoute s'avère parfois limitant. La question se pose sur quelles composantes de la communauté phytoplanctonique le zooplancton dominant se nourrit. La sélectivité de broutage d'*E. affinis* a été quantifiée à l'aide d'expériences d'incubation et des quantification de contenu pigmentaire à l'aide d'HPLC. *E. affinis* sélectionne des diatomées au sein de la communauté phytoplanctonique et en moindre mesure des cryptomonades. L'impact de la population d'*E. affinis* sur le stock de phytoplancton – et sur les diatomées dominantes- est < 4.5 % $jour^{-1}$, ce qui implique que dans le tronçon d'eau douce de l'Escaut le zooplancton n'est pas limité par la nourriture et ne présente pas de limitation pour le développement les niveaux trophiques supérieurs. Certains taxons phytoplanctoniques (chlorophycées, par exemple) sont apparemment stimulés en croissance par la présence d'*E. affinis* dans les bouteilles expérimentales et l'impact précis d'*E. affinis* sur le phytoplancton non-diatomées est moins clair.

L'activité de broutage du microzooplancton a également été testée avec des expériences d'incubation. Son impact sur la communauté phytoplanctonique est variable en intensité et en sélectivité, nécessitant plus d'expérimentation.

Mots clés: zooplancton, estuaire, Escaut, restauration, *Eurytemora affinis,* contenu pigmentaire digestif, HPLC, sélectivité trophique.

Role of zooplankton in a restoring estuary: the Scheldt

Abstract: The Scheldt is an estuary on way of recovery. The study concerns the ecology of the zooplankton community in the freshwater reach of the estuary. In parallel to water quality improvement, the copepod *Eurytemora affinis* has become dominant since 2007 and abundance of cyclopoid copepods has decreased. We tried to find out which environmental factors had caused these changes in the zooplankton community composition. The results of RDA and GLM analysis between the abundance of zooplankton taxa and the environmental factors showed a strong link between *E. affinis* abundance and the increasing O_2 concentration, but also the decreasing NH4-N concentration. The upstream Scheldt became permissive for *E. affinis* development as soon as oxygen concentration was above the threshold level of 4 mg L^{-1} and the NH4-N concentration remained below 2 mg L^{-1}. The cause of the decrease in cyclopoid abundance remains unclear.

The phytoplankton community of freshwater Scheldt is strongly dominated by diatoms, but the dissolved silica concentration could become limiting for their development. The question arises on which phytoplankton taxa the dominant zooplankton feeds.

Grazing selectivity of *E. affinis* adults and CV was measured by incubation experiments using natural Scheldt water and by gut pigment content quantification. Phytoplankton taxa concentration was quantified by HPLC. *E. affinis* selects diatoms and sometimes cryptophytes. The impact of the *E. affinis* population on the phytoplankton standing stock is < 4.5% d^{-1}, which means that the zooplankton community is not food limited and hence does not present a limitation to the development of higher trophic levels. The grazing activity of the microzooplankton community has also been measured by incubation experiments. Its impact on the freshwater Scheldt phytoplankton community is variable in intensity and in selectivity, and clearly needs further investigation.

Key words: zooplankton, estuary, Scheldt, restoration, *Eurytemora affinis,* gut pigment content, HPLC, trophic selectivity.


DOMINANT
CAUSE
RESTE
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QUESTION
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OXYG NE
PHYTOPLANCTON
ENVIRONNEMENTAUX
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ZOOPLANCTONIQUE
DIATOM ES
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EURYTEMORA
AFFINIS
RESTAURATION
AMONT
COMMUNAUT
CONCENTRATION
ABONDANCE
PHYTOPLANCTONIQUE
ESTUAIRE
IMPACT
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